# NIH Research ADVANCES

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# Contents


CLINICAL CENTER
NATIONAL INSTITUTES OF HEALTH

The complex human endeavor known as biomedical research found itself affected in the past year by two powerful currents for reform. One was economic, and had to do with the costs of applying the products of research to improve the public health. The other was the ethical movement that regards some of the processes and some of the products of research as creating social choices that can no longer be left to the experts. Science, nurtured on an internal ethic that insists upon quantitative and reproducible proofs for problem solving found itself—as it has before—being accused of withdrawing from some of the "impossible decisions" posed by the impingement of scientific or technological developments on society.

And there were other issues facing biomedical research as well, stemming in part from the very size of the national effort, in part from the intense public interest in that effort, and in part from the mechanisms and techniques of the biomedical research apparatus itself. A Presidential Panel, mandated by the Congress, was established last year to look into those issues.

Against this broad backdrop of societal, professional, and managerial issues, the National Institutes of Health moved during the past year in accord with its well established mission. NIH continued to nurture and sustain the system which has brought us to the present high levels of understanding and accomplishment.

The purpose of biomedical research is improvement in the well-being of man through greater understanding of the nature of life. It is a continuing quest for knowledge, an enterprise that depends more upon disciplined imagination and hard work than it does upon single creative strokes of genius or serendipity. Whether basic scientist, clinical investigator, or alert practitioner, the people involved today are highly specialized professionals, and they all contribute to the flow of new knowledge.

Basic research is rooted in biology, the discipline concerned with the fundamental processes shared by living things. Its thrust is toward reduction of these essential functions to chemical and physical terms and then re-ordering this information into rules and patterns that explain the difference between the living and the inert, the "normal" and the abnormal.

Although it is often described as basic research, the discovery of cycles that transmute energy, regulate metabolism, or the intricate mechanisms for transmitting and transcribing the genetic code is also "applied" research in the sense that it transfers to complex systems the techniques and descriptors of other sciences.

The search for knowledge involves successive amalgamation of new facts with old ones and continuing resynthesis of the whole. Although at any one time the applicability of much fundamental research necessarily remains obscure, there is a constant desire that any discovery shall have some practical importance. Scientists whose lives are spent working upon bacteria or plants are no less conscious of this than those who work on human problems.



Nothing that has been learned about life is more important than the revelation that the biological processes which determine the growth of an organism from conception, that program its eventual death and sustain life in the interim, appear to be so remarkably similar from bacteria to man. Myriad lower forms of life have much of the same biochemistry as does man and have perhaps retained superior features of certain common systems. This limited divergence of fundamental processes is the strength and the rationale for the concept of biomedical research as a continuum. There is very little that is learned about any form of life that is not ultimately relevant to man. And much of that still vast unknown that surrounds man must still be learned through study of the many other species upon earth. NIH has never felt it must apologize for its emphasis upon this level of study, nor explain the relevance of this approach to its mission.

There are undoubtedly some in biology who wish that the medical half of biomedical research, with its pressing social issues, could exist separately. But the linkage of biology to medicine is no less dissoluble than the union of biomedical research with health care. Understandably there is a practical limit to the public desire to be the patron of either biology or biomedical research as solitary entities.

There are more compelling intellectual reasons for these unions. Medicine has frequently been a tutor of biology. The steadily increasing capacity for reduction of certain diseases to molecular terms steadily enhances this cross-fertilization. There are now numerous examples of enzymes, of new functions for other proteins, and of metabolic pathways that were unsuspected or could not be proved in studies limited to normal organisms. The resulting surge in biomedical knowledge acquired in the past 30 years will stand as one of the greatest periods in



the history of science and probably of all human endeavor. The impetus of the NIH effort contributed to that advance.

In the realm of clinical investigation the singular national contribution to this world-wide jump in biomedical knowledge is worth noting. The generous level of American investment in all phases of research has been an essential catalyst to this supremacy in the coupling of medicine and biology.

Since the Second World War, the Federal Government has become the largest single purchaser of medical care. The role of Government has also changed toward assuming a responsibility for guaranteeing health as one of the civil rights. In the same years a change has come about, too, in the definition of health. More is implied than simply the diagnosis and treatment of specific diseases. Health has come to mean no less than some control of the number and fitness of children born into a society, a thorough understanding and improvement of the physical and social environment in which people live and work, the helping of each person to know something of this genetic constitution and how he might best modify his lifestyle to adapt to his environment and avoid disease, the restoring of function and adaptation when they fail prematurely, the easing of the discomfort of the incurably ill and aged, and the assuring that when life must end, it does so with dignity. The National Institutes of Health has contributed to the knowledge base on which this conceptual change has been developed.

The need for closing the translation gap that exists between biomedical research and the effective application of its discoveries is a prime issue facing the research community—and thus the National Institutes of Health—in the years ahead. An element of urgency is derived from a national determination, largely Government directed, to increase the cost-effectiveness and efficacy of health care. The importance of clinical trials in this regard is several fold. Such trials provide a reaffirmation of the necessity of the union of biological and medical research, while simultaneously they raise a question of how far resources can be deployed in matters of medical practice before the principal mission of discovery is imperiled. Thus they are one element in the complex question involved in the setting of boundaries of an agency whose central mission is research and in establishing priorities within that endeavor.

Clinical trials also compel better understanding of the apparatus that is supposed to provide for orderly transition of discoveries into the substance of medical care. The processes of validation and continuing re-evaluation do not always operate smoothly or even serially. The loose confederation of diverse talents and interests involved need better articulation. An essential first step will be recognition of collective responsibility for any gaps in translation that persist.

Some life scientists view large-scale clinical trials as a potential drain on the resources supporting all biomedical research. In the past, moreover, there has been criticism of the design and methodology of many clinical trials. But in

recent years there has been considerable growth in the knowledge of how to conduct clinical trials and how to evaluate their costs and efficacy. This has come at a time when biomedical research is being asked to become more involved in clinical interventions.

The effects of technology pose another series of questions for the research community. Modern science has often been accused not only of failing to consider the consequences of technology but of embarking upon developmental inquiry to the neglect of research directed

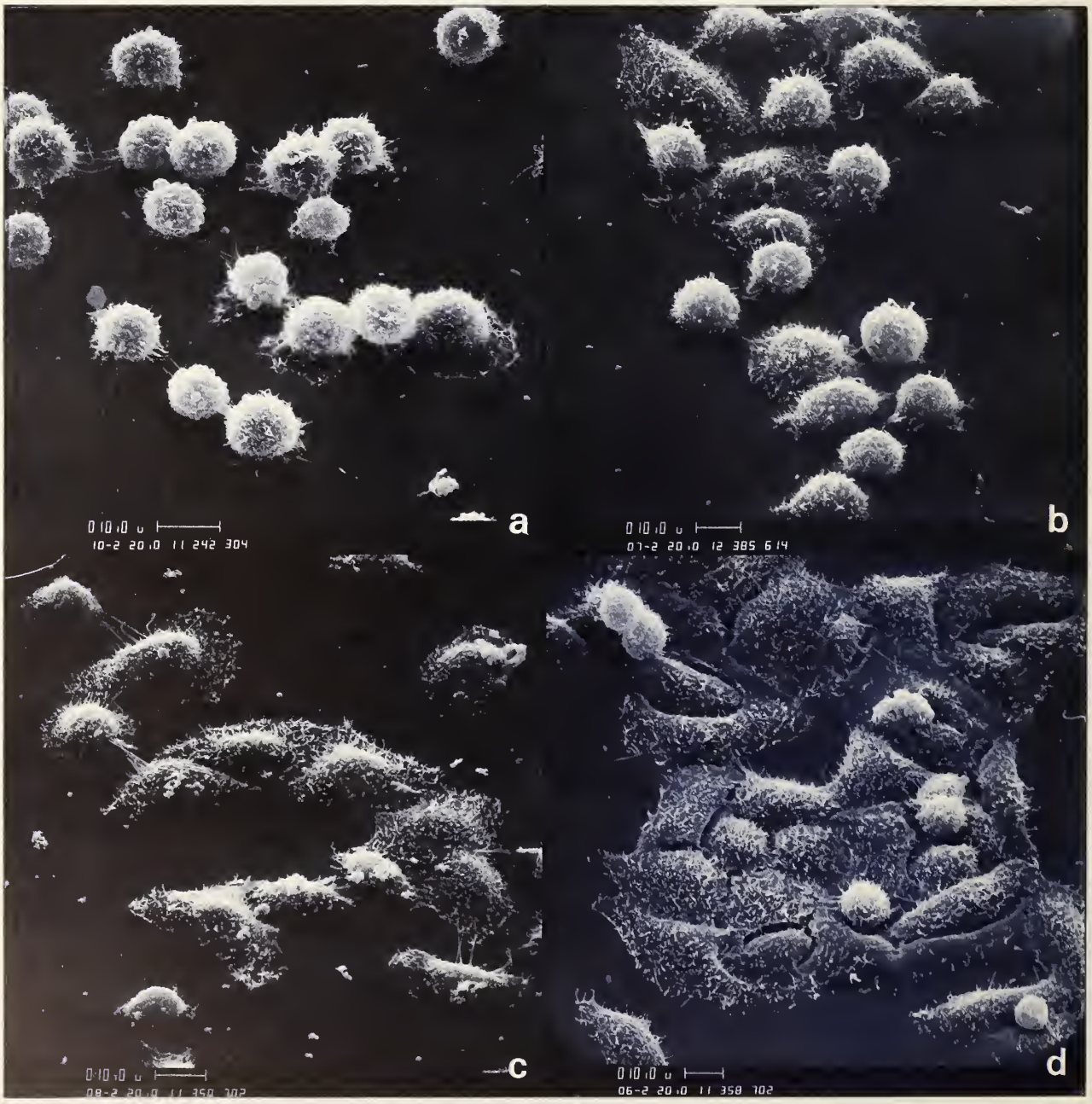
toward prevention or other long-range solutions. Priority selection is involved here—a complex, delicate problem.

How these and other challenges are met will to a large extent determine the future of biomedical research at NIH—and because NIH is so central to this endeavor nationally—in the Nation as a whole. Yet there can be no question that biomedical research can and will continue to be the instrument of discovery and still demonstrate its capability to keep pace with the momentum of the new priorities, to the ultimate benefit of society.

The pages that follow illustrate the scope and versatility of biomedical research today, and the promise of that new knowledge against which mankind's most ancient and implacable foes must inevitably yield.

When introduced into a new environment, isolated cells readjust to their normal shape. These cancer cells, seen under the scanning electron microscope, have been in culture for (a) 1 hour, (b) 8 hours, (c) 12 hours, and (d) 24 hours.





## Immunology is a Two-Way Street

Within the past year, immunologists have come to the realization that there are both positive and negative aspects to the body's immunological system; that the immune response is a two-way street where white blood cells can turn off, as well as stimulate, the production of protective antibodies.

One of the pioneering investigations of the dual role of T-lymphocytes—white blood cells that originate in the bone marrow as stem cells and are activated in the thymus gland (T) before becoming immunologically competent—was reported recently by National Cancer Institute scientists.

Dr. Thomas A. Waldmann and his associates found that "suppressor" T-lymphocytes apparently were responsible for reducing antibody production in patients suffering from a rare disease of the immune system called common variable hypogammaglobulinemia (CVH).

It was previously known that T-cells could function as "helpers" to stimulate the production of immunoglobulin by B-cells—the other type of lymphocyte also derived from bone marrow stem cells. Antibodies are made of immunoglobulins. T-cells are also involved in cellular immunity by directly attacking bacteria and other foreign agents in the body.

Experiments indicating the existence of suppressor T-cells in animals were reported in 1970 by Dr. Phillip J. Baker of the National Institute of Allergy and Infectious Diseases. Research since then at Yale and Harvard Universities and the National Institute of Arthritis, Metabolism and Digestive Diseases has provided support for the concept that a type of lymphocyte can exert a negative control of immune responses.

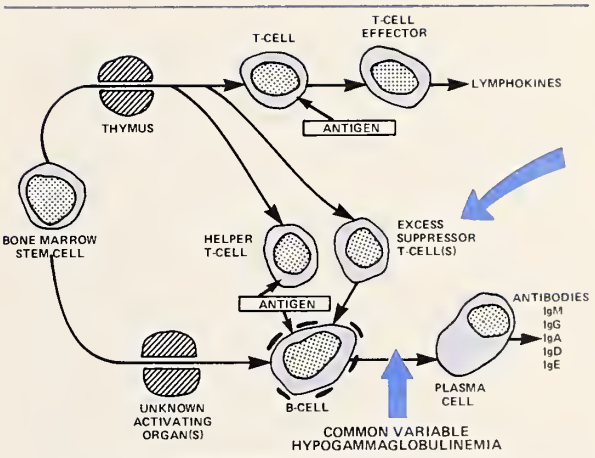
The NCI study has shown that suppressor T-cells have a role in human diseases as well. CVH patients have a deficiency of immunoglobulins. Dr. Waldmann and his colleagues found that the immune defect in some of these patients was caused by excessive levels of suppressor T-cells.

CVH patients are known to be prone to acute infections, such as pneumonia and respiratory inflammations, and have a much higher than average incidence of cancer, arthritis and other chronic diseases. About 50 CVH patients have been treated at the NIH Clinical Center; 13 of these formed the basis for the NCI study.

The scientists grew cultures of peripheral blood lymphocytes from the 13 patients and compared the immunoglobulin production with that of lymphocytes from normal volunteers. Despite chemical stimulation by pokeweed mitogen, lymphocytes from the CVH patients produced from 16 to 37 times fewer antibodies than normal lymphocytes.

Further measurements of immunoglobulin secretions when lymphocytes of five of the patients were co-cultured with normal lymphocytes showed a depression in antibody production of more than 80 percent, indicating that the CVH lymphocytes were acting as suppressors. A






comparable depression was noted when purified T-cells from patients were co-cultured with normal lymphocytes.

Immunoglobulin production remained unaffected when normal lymphocytes were cultured in serum taken from CVH patients. The NCI scientists concluded that T-cells in CVH patients were suppressing the maturation of B-cells into fully competent antibody-producing cells.

In addition to CVH, T-cell anomalies may be implicated in bone marrow cancers and benign and malignant tumors of the thymus. Systemic lupus erythematosus (SLE), an autoimmune disease in which antibodies are created against the body's own tissues, might be the result of dysfunction in both helper and suppressor T-cells.

## Cancer Prevention by Antioxidants

A growing number of compounds, of widely differing structure and chemical characteristics, have been identified as carcinogens—cancer-causing agents—in animal tests. About 30 chemicals have been associated clearly with cancer causation in man, but scientists now suspect that chemical carcinogens occur more widely in the environment than heretofore believed.

Cancer might be prevented by eliminating human exposures to carcinogens wherever possible. In addition, however, scientists have been seeking ways to block the action of carcinogens or reverse the cancer-inducing process at an early stage of cell transformation. This approach has been spurred in recent years by evidence that many different chemical carcinogens may act at the molecular level through similar mechanisms. Research on an effective counteracting agent thus might lead to preventing cancers caused by a broad range of carcinogens.



Scientists in several laboratories have been exploring the carcinogenesis-inhibiting properties of compounds that act as antioxidants. Antioxidants block chemical reactions of substances with oxygen and are used to prevent deterioration of foods, rubber and other materials. Dr. Lee W. Wattenberg, an NCI grantee at the University of Minnesota Medical School, Minneapolis, has reported that antioxidants significantly decrease cancer formation in mice and rats when administered either before or with chemical cancer-causing agents.

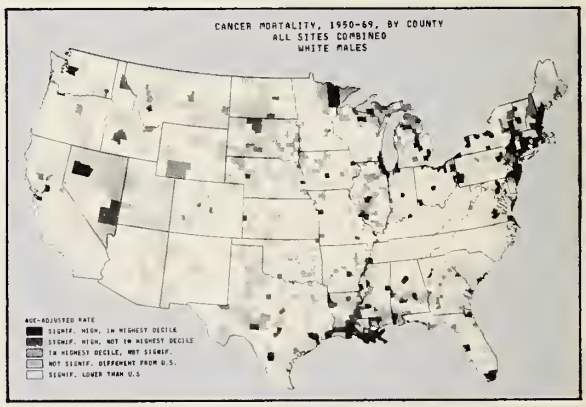
Dr. Wattenberg found cancer inhibition with three widely used antioxidants: butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), and ethoxyquin. Recently he has extended his findings to antioxidants that are sulfur-containing compounds with molecular structures different from the earlier compounds.

Past studies have shown that the carcinogen 7,12-dimethylbenz(a) - anthracene (DMBA) caused mammary (breast) cancers when given by oral administration to female rats. Another carcinogen, benzo(a)pyrene (BP), caused a high incidence of cancers of the forestomach when included in the diet of mice. When Dr. Wattenberg added one of the sulfur-containing antioxidants—disulfiram or dimethylthiocarbamate—to the diet of rats given DMBA, he observed a decrease in the number of rats with cancer and the number of tumors per animal. An additional sulfur-containing compound, benzyl thiocyanate, had a similar effect. Disulfiram also inhibited stomach cancer formation in mice given BP.

Dr. Wattenberg's research has broadened the range of antioxidant compounds which inhibit chemical carcinogenesis in experimental systems. The data suggest that different chemicals with a common function can act to interfere with cancer causation. The results are consistent with the hypothesis that many carcinogens may act via common mechanisms.

## Mapping Cancer

Many scientists believe that environmental factors are associated in some complex fashion with the development of a large majority of human cancers. Chemicals, radiation and possibly viruses may interact with each other and with factors of individual susceptibility over many years of exposure or latent development to produce any of the more than 100 forms of cancer. Such factors as diet, occupation, the air and water environments, smoking and the variations among lifestyles may be involved.




The biochemical and molecular details of cancer causation are poorly understoood. Only 30 or so chemical substances have been identified clearly as causes of human cancer. The belief that environmental chemicals play a major role in cancer comes from epidemiological studies and animal studies. Population studies have demonstrated higher-than-average cancer risks in certain occupational groups, changes in cancer risks among migrants from one country to another, and striking variations in cancer death rates from one part of the world to another. By studying the changes and differences among countries, scientists have identified many clues to the causes of cancer.

However, little has been known of the variation in cancer rates within a single country. A new National Cancer Institute mapping study, *Atlas of Cancer Mortality for U.S. Counties: 1950-1969,* is the first systematic analysis of geographic variation of cancer that has been done in the U.S. at the county level. The authors of the *Atlas* are Dr. Thomas J. Mason, Frank W. McKay, and Drs. Robert Hoover, William J. Blot, and Joseph F. Fraumeni, Jr.

The maps are based on average annual cancer death rates (deaths per 100,000 population) computed after tabulation of cancer deaths in the U.S. during the 20-year period 1950-1969.

Data from death certificates for this period were provided on race, sex, age, type of cancer, and place of usual residence, by HEW's National Center for Health Statistics.

A basic premise of the mapping study is that similar geographic patterns for both men and women suggest that common environmental factors may contribute to the causation of a particular cancer, but that markedly different patterns between the sexes suggest the effects of occupational factors instead.

For several types of cancer, the NCI scientists found that the geographic pattern was consistent with what is already known. For example, melanoma (a rare skin cancer) occurred predominantly in the southern U.S., where the effect of overexposure to sunlight would be most marked. Above-average rates of cancers of the colon and rectum were found in both sexes in the Northeast and in urban areas near the Great Lakes. Low rates occurred in the central U.S. and in the South. This pattern of high and low rates for both sexes is consistent with the theory that an environmental factor common to men and women may play an important role in colon and rectal cancers.

Breast cancer showed a similar pattern, suggesting that this cancer may have an environmental factor in common with cancers of the large intestine.

In the Northeast, the scientists also found higher-than-average rates of cancers of the esophagus, larynx, mouth and throat, and bladder, but only in males, suggesting the influence of occupational factors. Stomach cancer rates were high in both sexes in the North Central States, corresponding closely with the geographic concentration in these areas of persons with ancestors from Austria, the Soviet Union, and Scandinavia, where stomach cancer rates are higher than in the U.S.

High death rates for lung cancer were found in urban centers and along the Gulf Coast from Texas to the Florida Panhandle. Of Louisiana's 64 counties, 13 are in the top one percent of rates for the entire U.S., as are an additional seven counties along the Gulf Coast and along the Atlantic Coast from northern Florida to Charleston, South Carolina. This pattern suggests that environmental factors, in addition to cigarette smoking, may be contributing to lung cancer deaths in these predominantly rural and seaport areas.

Cancer death rates were mapped so far only for whites. Even among this large segment of the U.S. population, the NCI scientists had to use a basic unit larger than a county, called a state economic area, to map the less common cancers. For their study on nonwhites, the scientists are modifying the mapping technique even further.

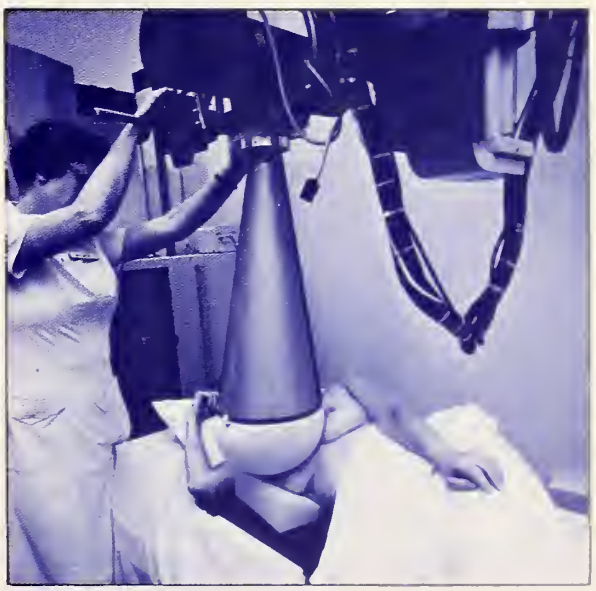
### Early Detection of Breast Cancer

After superficial skin cancer, cancer of the breast is the most common form of cancer in U.S. women. The key to successful treatment of breast cancer is early detection before the cancer has spread beyond its initial site.



With that goal in mind, a breast cancer screening program has been established as part of the Cancer Control Program. Preliminary findings from the program have indicated improved capabilities to detect cancer earlier with a combination of detection techniques.

With early diagnosis, 8 out of 10 women who develop breast cancer will survive at least 5 years. The goal of early diagnosis is to find breast cancer while it is still a small and localized tumor, before it has a chance to spread to the axillary nodes (the lymph nodes in the armpit). Once the cancer involves the axillary nodes, the 5-year survival rate will drop from 80 percent to about 45 percent.



Screening for breast cancer (by xeroradiography)

Jointly with the American Cancer Society, the National Cancer Institute has established 27 breast cancer detection demonstration projects across the United States. Each of the projects will screen 10,000 women between the ages of 35 and 75. The women who volunteer for the screening

program are given a physical examination, an X-ray examination by film mammography or xeroradiography, and a thermogram. A thermogram maps temperature patterns in the breasts and is based on the premise that a tumor will appear as an area that is warmer than the normal breast tissue.

Screening data have been collected for nearly 175,000 women. A total of 671 cancers were found, only 23 percent of which involved the lymph nodes. Normally, in an unscreened population, more than 60 percent of the breast cancers found have spread to the axillary nodes.



The women in whom breast cancer is found will be followed for several years after they undergo treatment. The follow-up will reveal whether the improved rate of early detection leads to the desired increase in rates of cure and long-term survival.

### Genetic Trait Provides Clue to Breast Cancer Risk

A genetic trait indigenous to the Mongolian race may provide a clue to the decreased risk of breast cancer among oriental women, according to recent findings of Dr. Nicholas L. Petrakis of the G. W. Hooper Foundation at the University of California, San Francisco.

Epidemiologists have long noted that breast cancer is relatively uncommon among Japanese, Chinese and American Indian women. In contrast, it is the leading cause of cancer death among U.S. Caucasian women. Aside from their low breast cancer rate, most orientals have dry, scant ear wax (or cerumen), whereas most Western whites and blacks have a wet, sticky type of ear wax. Dr. E. Matsunaga, a Japanese geneticist, showed that this trait is hereditary and is controlled by a single pair of genes. Because the wet type of wax is genetically dominant over the dry type, orientals inbred with Caucasians will have wet cerumen.

An association between ear wax and breast cancer is not as unrelated as it might seem at first. The glands of the ear that secrete wax, and the glands of the breast that secrete fluid and milk are similar in their cellular characteristics. Both are modified sweat glands of the apocrine type. Apocrine refers to the way secretory cells release their product by pinching off part of the cytoplasm. Sweat glands in the axilla (armpit), groin, genital and anal areas are apocrine glands, also. All three types of modified sweat glands begin to enlarge at puberty, and they have biochemically-similar secretions that contain a high concentration of lipids. Dr. Petrakis examined the type of ear wax in a small group of Japanese women in California, and found that 29 percent (9 of 31) of women with proven breast cancer had wet cerumen. Only 17 percent (9 of 52) of healthy Japanese women had wet ear wax. These findings, while not statistically significant, suggested that wet ear wax may be related to a risk factor for breast cancer and prompted a wider-based study.

In more recent studies, Dr. Petrakis examined the ability of women of different races to secrete breast fluid. Women, regardless of age, whether they have ever been pregnant or nursed a baby, can secrete a fluid from the apocrine ducts of the breast. In this study, fluid was obtained from the ductal system by a specially-designed breast pump that exerts a mild vacuum, only moderately greater than that developed by nursing infants.

Women living in the San Francisco Bay area who had no evidence of breast disease served as the sample: 236 were Chinese, 225 Caucasian, 71 Mexican-American, 37 Negro, 27 Japanese and 10 Filipino.

Overall, breast fluid was obtained successfully from 48 percent of the 606 women. Fluid samples were obtained most often from Caucasians (70 percent success) and least often from Chinese (24 percent success). Successful aspirations declined in women over 50 years of age in all racial groups, but distinctly so in orientals. Chinese and Japanese women with dry-type cerumen had a lower percentage of successful aspirations than those with wet wax.

The greater frequency of secretory activity among Chinese and Japanese women with wet cerumen suggests that a group of genes may regulate development and functioning of the apocrine glands. The finding is supported by a Japanese study showing an association between dry cerumen type and decreased secretions of axillary apocrine sweat glands. Further, this genetic variation in the apocrine system may influence susceptibility to breast cancer.

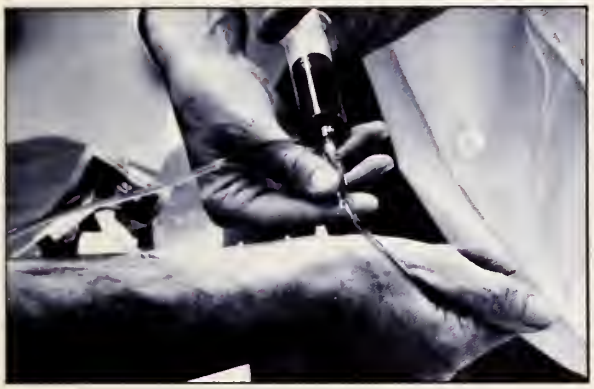
### Adjuvant Chemotherapy for Solid Tumors

Adjuvant chemotherapy, the use of drugs early in the course of cancer treatment, is improving the outlook for patients with breast cancer and a certain form of bone cancer.

Surgery has been the traditional first line of defense against solid tumors, those arising in specific tissue or organ sites. More recently,

physicians have used radiotherapy in the initial treatment of certain cancers. When the tumor recurred or metastasized, a second line of defense—including drugs as well as additional surgery or radiation—would be used. This approach has failed to improve survival of many cancer patients, despite the availability of more active drugs and improved surgical and radiation techniques.

At least two important trends are beginning to change the therapeutic climate: A better understanding of the natural history of various



Adjuvant Chemotherapy

types of cancer, and the acceptance of the use of anticancer drugs, singly or in combination, as safe and effective in patients with early disease.

Physicians have a better understanding of the behavior of certain cancers. They know, for example, that a patient with osteogenic sarcoma, a cancer of the bone, has an 80 percent chance of recurrence with lung metastases within a year following surgery. A woman who has had a cancerous breast removed and has four or more positive axillary (armpit) lymph nodes at the time of the initial treatment has a 90 percent chance of recurrence within 10 years. This means that cancer is present beyond the original tumor site at the time of operation.

Physicians have also learned more about the use of anticancer drugs. They know how to handle the toxic side effects, how to regulate dosage and scheduling, and how to use drugs in effective combinations. They also appreciate that drugs work better when the number of tumor cells is small, and that recurrent tumors are especially difficult to treat.

Because the greatest potential for cure of any cancer is at the time of the initial treatment, oncologists have adopted a new strategy for treating solid tumors—adjuvant chemotherapy, the use of effective drug therapies as an adjuvant to the primary treatment for those patients at a high risk of recurrence. Studies with two types of cancer in the past several years show promise for this approach.

One was a study of women with breast cancer, conducted by cooperating physicians at 37 U.S. and Canadian hospitals with support from the National Cancer Institute. L-Phenylalanine mustard (L-PAM) was given in pill form to 103 women with positive axillary nodes as an adjuvant to breast surgery. A randomly selected control group of 108 patients received an inactive placebo instead of the drug. The preliminary results were particulary striking in premenopausal women: Cancer recurred after 2 years in only 1 out of 30 patients receiving L-PAM, compared with 11 recurrences among 37 patients receiving surgery but no drug. For postmenopausal women, the recurrence rates were also reduced in the group treated with L-PAM, but not as markedly.

In another NCI-supported study being conducted by Dr. Gianni Bonadonna at the National Cancer Institute in Milan, Italy, preliminary reports have indicated that a three-drug combination of Cytoxan, methotrexate and 5-fluorouracil (CMF) may be even more effective than a single drug. Only 2 percent of 250 breast cancer patients who received the combination chemotherapy relapsed, whereas 12 percent of 199 patients in a control group who received no further treatment after surgery relapsed. At the time of the report, half of the treated group had been followed for 1 year or longer after surgery. Half of the control group had been followed for at least 9 months.

A second series of adjuvant therapy studies involved patients with osteogenic sarcoma, a cancer arising in the bone, cartilage and fibrous tissue. Although this form of cancer is relatively rare in mature adults, it is the third most common type of cancer in children and young adults. The ability of two anticancer drugs—methotrexate in high doses and Adriamycin—to prolong survival of patients with recurrent osteogenic sarcoma, led to trials of the drugs as adjuvants to surgery.

Dr. Norman Jaffe and coworkers at the Children's Cancer Research Foundation in Boston have pioneered the use of high doses of methotrexate with citrovorum factor. Methotrexate is a folic acid antagonist that interferes with an enzyme critical for the synthesis of nucleic acids and proteins. In the high dose treatment regimen (low doses are ineffective), potentially lethal doses of methotrexate are

given, followed in 2 hours by citrovorum factor (folinic acid) which nullifies the toxic action of methotrexate by overcoming the enzyme blockade. The antidote literally "rescues" the patient from an otherwise fatal dose of anticancer drug.

Of 19 patients treated with this regimen, only 2 have relapsed with lung metastases. They have been followed 1 to 22 months. Using historic data, the Boston group has calculated that 12 patients would be expected to have relapsed.



Dr. Engracia P. Cortes of Long Island Jewish Medical Center, New Hyde Park, New York, and other members of the Acute Leukemia Group B, an NCI cooperative clinical group, have reported encouraging preliminary results using Adriamycin. Of 30 osteogenic sarcoma patients who received a full course of the drug, only 3 have relapsed after as long as 2½ years. Ten patients have been without evidence of disease for more than 1 year after cessation of chemotherapy.

Unfortunately, both Adriamycin and methotrexate have limitations. Several investigators have noted that tumors become resistant to methotrexate. Adriamycin can only be given to a certain total dose because the drug has a potential cardiotoxicity that can result in congestive heart failure.

With these limitations in mind, investigators are now using the two regimens in combination and have designed complex schedules of administration to stretch out the effective times of both drugs. Two other drugs, vincristine and Cytoxan, have been added. Results from these studies, still very preliminary, seem to indicate that the combination therapy will further improve the results of adjuvant chemotherapy for osteogenic sarcoma.

### Estrogen Receptors in Breast Cancer

Human breast cancers have long been regarded as falling biologically into one of two groups—those generally responsive to hormone therapy in this instance means removing the sources or counteracting the effects of the patient's own hormones.

Even advanced cancers that are hormone dependent will usually respond favorably to hormone therapy. Until recently physicians had no way of judging whether a cancer was hormone dependent until they had carried out one or more courses of treatment. As a result the use of hormone therapy for a nondependent tumor might seriously delay the use of other types of therapy, whereas the use of other treatments for hormone-dependent cancer might deprive the patient of a period of hormonally induced regression.

Development of a laboratory test to identify hormone-dependent cancers has been under active investigation. For almost a decade scientists have been studying certain proteins that bind female hormones called estrogens in breast cells as possible indicators of the estrogen dependency of these cells. These proteins are called estrogen receptors (ER).

Apparent success was reported by several investigators, including Dr. Elwood V. Jensen of the University of Chicago, working under National Cancer Institute funding. The Chicago scientist found in 1970 that the cancers in five of seven women who had receptors responded to hormone therapy. Among women whose breast cancers lacked the estrogen receptor, only one of 19 responded favorably to hormone therapy.

Following Dr. Jensen's announcement, other laboratories began working to refine the detection procedure for ER and to confirm and extend the correlation with response to treatment. In July 1974, the NCI Breast Cancer Task Force convened a workshop at which investigators from eight countries discussed the techniques and potential merit of the estrogen receptor assay. The consensus of these scientists was that although this procedure could not easily be used on a widespread basis, clinical studies had shown the test to be a valuable prognostic indicator of whether breast cancer would respond to hormone therapy.

Overall, about 50 percent of the several hundred advanced breast cancers reported at the workshop showed an estrogen-binding ability. Of this group, almost 60 percent responded to hormone therapy. The studies further demonstrated that breast cancers lacking estrogen receptors would seldom respond to hormone therapy and should be treated instead with non-hormonal anticancer drugs.

Estrogen receptors or "estrophiles" are protein molecules in the cells of some breast cancers which attach to, or bind, estrogen compounds. These estrogen receptors are very selective in that they are specific for estrogens and will not bind similar materials such as testosterone, a male hormone, and progesterone, another female hormone.

An ER determination is made on cancerous tissue from a breast that has been removed surgically or on a sample of tissue from a cancer that has metastasized elsewhere in the body. If

the tissue proved to be "ER positive," any secondary cancer might be hormone dependent. Hormone therapy is usually reserved for the patient whose cancer recurs after the breast with the primary tumor has been removed.

For premenopausal women, the ovaries are removed surgically to deny the advanced cancer its source of estrogen. In a woman past the age of menopause, one form of hormone therapy consists of surgically removing other glands that control the production of estrogen: the adrenal glands, which are situated atop the kidneys, or the pituitary gland (hypophysis), which is located at the base of the brain. Hormones, including estrogens and androgens, are actually given to the patient in large doses in another form of hormone therapy. The doses are at least 10 to 20 times those used to control symptoms of menopause.

Scientists are working to simplify the procedure for determining whether a breast cancer contains estrogen receptors and to identify receptors for other hormones.

### CANCERLINE and the Cancer Data Bank Program

CANCERLINE (Cancer Information On-Line) is one of a number of information services developed by the International Cancer Research Data Bank (ICRDB) Program of the National Cancer Institute. By early 1976, CANCERLINE had more than 50,000 abstracts summarizing the results of cancer research projects that have been published in nearly 3,500 leading biomedical journals around the world since 1963.

Results of research on cancer therapy and the causation of cancer make up the major portion of the CANCERLINE data base. However, studies of cancer epidemiology, pathogenesis, biochemistry, immunology, and other cancer biology are also included. Each year, more than 15,000 new abstracts of published cancer research results will be added to this data base.

Scientists and clinicians affiliated with approximately 450 different research centers and other organizations throughout the United States (and, increasingly, in other countries) can easily retrieve information about a specific topic from CANCERLINE through on-line terminals linked by telephone lines to the computer system of the National Library of Medicine (NLM). Any combination of words or terms that appear in the title, text, index, or author sections of each record can be used to narrow the search to a very specific area.

The retrieved abstracts can be printed locally on a simple automatic typewriter or can be viewed on a TV-like screen. When many abstracts are retrieved, they can be printed at the NLM computer center and mailed to the researcher, usually within 24 hours. Scientists lacking easy access to a local computer terminal can usually call a search analyst in a nearby biomedical library and request a search.

In addition to abstracts of published results, a second CANCERLINE data base, available early in 1976, has approximately 10,000 descriptions of



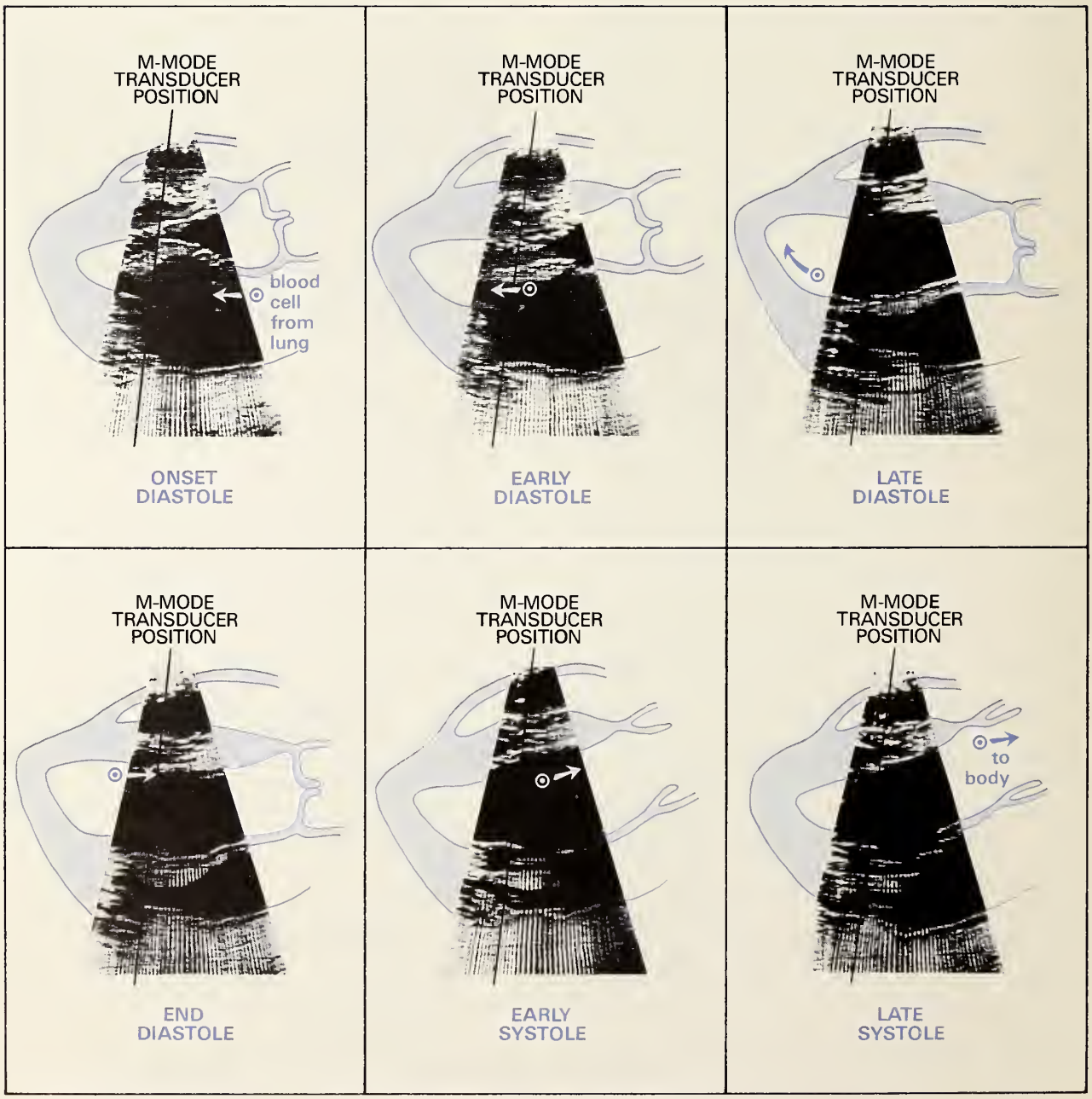
CANCERLINE terminal gives scientists rapid scanning of cancer research abstracts.

current, on-going research projects, including nearly 1,000 summaries of research protocols for controlled clinical trials in the treatment of cancer patients.

These two CANCERLINE data bases will help cancer researchers and clinicians locate data useful to their work. Another valuable information service made possible by CANCERLINE is a system for Selective Dissemination of Information which is used to send a steady stream of new abstracts and project descriptions in a narrow subject area to individuals working in that specific area. This type of alerting service, along with technical bulletins and other dissemination projects, are designed to promote the rapid exchange of information between cancer researchers and clinicians.

Two-dimensional echocardiography is a painless, safe, noninvasive technique for visualizing the heart and great vessels. In this technique, an ultrasonic beam is directed through the intact chest at the heart. Portions of the beam are reflected from the heart and from blood-tissue interfaces within it. Returning echoes are converted into two-dimensional images, permitting detection of structural defects. The below pictures show normal heart structure during the pumping cycle.





## Research on Cooley's Anemia

In their research on Cooley's anemia, sickle cell anemia, and other hereditary disorders affecting the production of hemoglobin by red blood cells, National Heart and Lung Institute scientists are using a powerful new technique called cell fusion. The research is being performed by Drs. Albert Deisseroth, A. W. Nienhuis, W. F. Anderson, Raymond Velez, Jane Barber, Judith Kantor, Alan Steggles, and Golder Wilson.

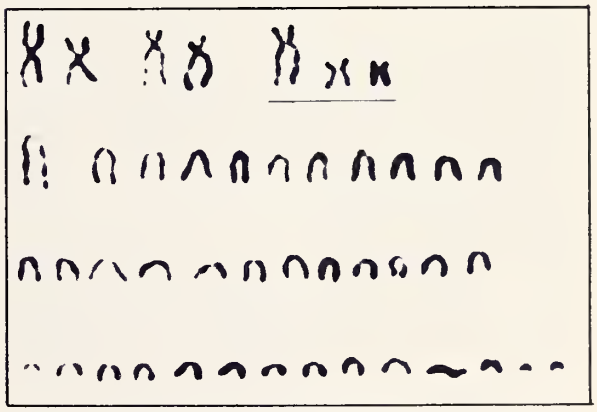
The fusion of hemoglobin-producing cells from different species (mouse and human, hamster and human, etc.) results in hybrid cells containing various mixes of the genetic material originally present in both parent cells. This material, DNA, is carried by genes located on structures called chromosomes in the cell nucleus. In some of these "matings" many of the normal human complement of 24 chromosomes are excluded from the resulting hybrid. In fact, the fusions of mouse and human erythroid cells has yielded some hybrid strains containing only a single human chromosome in company with a full complement of mouse chromosomes. The single human chromosome incorporated may vary from one hybrid strain to the next; but the fusion technique effectively isolates that chromosome for studies of what genes it carries, their locations on the chromosome, or how the genetic blueprints carried by the DNA of the genes are translated (via RNA) into the structural proteins, enzymes, and other substances produced in the cell.

Cooley's anemia, also called beta thalassemia, is a serious hereditary hemoglobin disorder that affects chiefly Greeks, Italians, and other Mediterranean peoples and their descendants. The thalassemias comprise a family of diseases whose prevalence in the U.S. is not known with certainty, but worldwide, these genetic defects are very common and the severe forms of the thalassemias are a major health problem.

Earlier NHLI studies disclosed the genetic defect underlying Cooley's anemia. In the normal synthesis of hemoglobin (the oxygen-carrying substance of the red blood cell or its precursors), eight "sub-units" are required. Four of these are molecules of an iron-containing substance called heme. Assembled in the proper alignment the four heme molecules act as the prosthetic group, or "business end" of hemoglobin. The other four components are protein chains called globins. Two are identical proteins called alpha chains; the other two are identical proteins called beta chains. Alpha and beta chains differ from each other in molecular weight and also in the amino-acid sequence of their respective chains.

Normally, alpha and beta chains are synthesized at the same rates by hemoglobin-producing cells. In Cooley's anemia, alpha chains are produced at the normal rate, but beta chain production is abnormally slow. As a result, excess alpha chains pile up in the red blood cell, then precipitate, "crippling" the red blood cell and causing it to be destroyed long before it has lived out its allotted span. This is the basis of the anemia that can now be corrected only by periodic transfusions.





Human chromosomes (underlined) among others of mouse origin in a hybrid cell produced by cell fusion methods.

The NHLI studies established that the subnormal production of beta chains was due to a scarcity of beta globin messenger RNA (beta-mRNA). Produced by DNA in the nucleus, beta-mRNA carries the "blueprint" for the assembly of the beta chain. The limited amount of beta-mRNA in the cells from patients with Cooley's anemia is structurally normal, the NHLI studies showed, and so are the beta chains synthesized by the beta-mRNA. There is simply too little beta-mRNA to keep pace with the production of alpha chains by alpha-mRNA.

The basic flaw of Cooley's anemia, then, appears to be faulty regulation in the synthesis of a gene product. Cell-fusion techniques offer a promising means of zeroing in on mechanisms involved in globin-mRNA synthesis by DNA and on factors that may stimulate or inhibit mRNA output in red-cell precursors and thereby affect hemoglobin synthesis in the circulating red blood cell.

Studies thus far with mouse/human erythroid hybrids have yielded strains that 1) produced neither mouse nor human globins; 2) produced

mouse, but not human globins; 3) produced mouse globins and human beta-chain globin, but not human alpha globin. This last hybrid indicates that the genes producing alpha and beta globin mRNA are located on different human chromosomes.

Certain of the hybrid cells have been shown to contain both mouse and human globin genes, but these genes are not expressed in mRNA production or hemoglobin synthesis. Thus regulatory factors are operative in them.

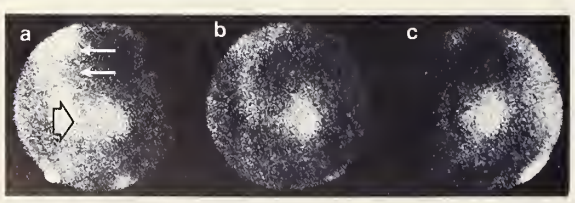


The human erythroid cell contains a number of bi-armed chromosomes, and it appears that two of these carry the genes for alpha and beta globin mRNA since expression of the human globin genes has only occurred in hybrid cells which contain at least two or three human bi-armed chromosomes.

Continued research on the genetic flaw in Cooley's anemia may lead to treatment methods more direct and effective than periodic transfusions. Transfusion temporarily restores the oxygen-carrying capacity of the blood of patients with Cooley's anemia; but the continuous destruction of beta-globin deficient cells and reduced life of transfused red cells result in the accumulation in various tissues of excessive iron, a condition called hemosiderosis. The effects vary from one tissue to the next, but hemosiderosis is a leading cause of disability and death in Cooley's anemia. Chelating agents, which combine with iron to form complexes more readily excreted by the kidneys, hold promise for the treatment of hemosiderosis, but results thus far have been only modestly successful.

### Bone Scanning Agent Reveals Infarcts

Now that it is becoming possible for cardiologists and heart surgeons to save heart muscle stricken by coronary occlusive disease, there is an urgent need for better ways of visualizing the injured myocardial areas.




Positive (4+) scintigrams of LP with lateral wall infraction. (a) Anterior view, small arrows point to sternum with myocardial infarction indicated by open arrow. (b) 45° left anterior oblique view. (c) Left lateral view.

A heart attack always involves some localized, fairly rapid heart muscle death, or myocardial infarction. This acute emergency happens when the occlusive, or closing-off, process in one of the coronary arterial branches culminates so suddenly and completely that the local myocardium—the muscle area that was fed by the occluded coronary branch—stops contracting and dies over a period of hours, to be replaced over a period of weeks by a scar, or healed infarct.

But the disability and pain of the underlying occlusive process in coronary disease can also develop quite gradually, and there is always some period of myocardial ischemia, or oxygen starvation, before the muscle cells actually die, which varies from a few hours in acute heart attack up to years in cases of angina pectoris, or chronic coronary insufficiency.

In recent years it has become possible to save ischemic myocardium or relieve angina pectoris in many coronary patients by surgically bypassing the occluded coronary segment and supplying the threatened heart muscle through a blood vessel graft. Also, a new idea of utmost importance now being explored is that the size of the infarct which would otherwise result from an acute coronary occlusion may be greatly reduced, and vitally needed working myocardium saved, by the actions of certain drugs and other measures taken during the acute phase of the heart attack. Central in this concept is a new realization that the size of the myocardial infarct is not really determined at the moment of the coronary occlusion; that the fate of the stricken muscle segment remains largely uncommitted, hanging on a balance of oxygen supply and demand which can be favorably influenced for hours after the coronary is occluded.

Thus, the need is growing for better non-invasive techniques of visualizing acute myocardial infarctions so as to determine their position and extent. Various methods have been developed in which the injection into the coronary circulation of diverse substances with radioactive labels yields scintigrams, or heart films of some diagnostic value. The limitation of most of these methods is that they label the normal heart muscle and thus yield a negative picture, presenting the injured portion as a void.

NHLI grantees Drs. Robert W. Parkey, Frederick J. Bonte, and James T. Willerson at the University of Texas Southwestern Medical School in Dallas reasoned that certain Technetium-99m/$^{99m}$Tc) labeled phosphate agents in common clinical use for bone scanning might serve. Other scientists had reported a tendency for calcium to

concentrate in damaged myocardial cells, and the bone scanning agents have a particular affinity for calcium. With one of the bone scanning agents, $^{99m}$Tc stannous pyrophosphate, injected intravenously, they were able to clearly visualize as "hot spots" the areas of acute myocardial infarction, first in dogs and then in human patients. The technique was found to be simple, non-invasive and safe, as well as relatively inexpensive.

"The technique," Dr. Parkey reports, "appears to represent a valuable tool not only for establishing the presence or absence of acute myocardial infarction, but for estimating the size of the area damaged by it and hopefully the effect of various therapeutic measures on the size and progression of myocardial infarcts."

### Metabolism of Ischemic Heart Muscle

When heart muscle is deprived of an adequate blood supply (a condition called myocardial ischemia), curtailment of its normal allotment of essential nutrients and oxygen alters the metabolism of affected areas of heart muscle and may result in serious, even lethal disturbances in heart performance.

The depth and permanence of these alterations depend on the severity and duration of the blood deprivation. There is also new evidence that the adverse effects of ischemia on heart muscle can be largely mitigated or even reversed by timely medical interventions.

The primary cause of myocardial ischemia is atherosclerosis blocking the coronary arteries that nourish the heart muscle itself. Coronary atherosclerosis usually strikes in the form of angina pectoris (chronic chest pain, especially during exertion), acute heart attack, or sudden cardiac death. In each instance, the culprit is heart-muscle ischemia.

Ischemic heart disease afflicts an estimated four million Americans and causes nearly 700,000 deaths each year. About half of these deaths occur before the victim can be hospitalized. Hence NHLI is vitally interested in the metabolic effects of ischemia in heart muscle, their resultant effects on heart performance, and any means of ameliorating these effects, so as to salvage threatened heart muscle and forestall crippling or fatal complications.

The biochemical sequence of events in ischemic heart muscle is thought to proceed as follows:

1) Oxygen deprivation causes affected areas of heart muscle to switch from aerobic to anaerobic processes of generating energy. Aerobic processes, carried out in cellular organelles called mitochondria, can convert almost any metabolic fuel - fat, protein, or carbohydrate - to ATP, the chemical power source for virtually all energy consuming processes in the body. The anaerobic process can burn only glycogen (the storage form of glucose) and is much less efficient in generating ATP. Moreover, it results in the accumulation of lactate, which the heart muscle cell cannot burn without oxygen.

2) As anaerobic metabolism proceeds, available stores of glycogen dwindle, as does the supply of ATP. The accumulation of lactate increases intracellular acidity, which is believed to trigger other adverse metabolic alterations. These may include the denaturation of proteins and enzymes in heart muscle cells and the activation of lysosomes, intracellular structures rich in protein-digesting enzymes that may act as an intracellular self-destruct mechanism.

Dr. Marlene A. DeLuca and Dr. John Ross, Jr.

3) Active transport mechanisms, by which the cell maintains its internal environment, begin to fail and the cell membrane becomes "leaky," allowing enzymes and other cellular components to escape. It also opens the door to passive invasion of the cell by ions and other substances whose concentrations are normally closely regulated by active transport mechanisms.

4) At some point, the seriously damaged cell passes the point of no return and perishes. What this point is, is not known with certainty nor is it clear which metabolic derangements are predominantly responsible for its demise.

At the University of California at San Diego, NHLI grantees M. J. DeLuca, J. S. Ingwall, and John Ross, Jr., are studying the effects of oxygen and nutrient deprivation in cultures of embryonic heart cells from birds and mice. These apparently behave very much like human heart cells *in vivo* and provide a "clean" system for studying intracellular metabolic reactions under

precisely controlled conditions.

The studies are exploring the relationship between intracellular ATP depletion and decreased viability; factors affecting membrane permeability and the cellular "leakage" of constituents such as enzymes; ways to restore flagging energy-generating processes in ischemic cells; protein synthesis under varying conditions of oxygen or nutrient deprivation and ways to stimulate repair processes in damaged cells.

The scientists are also seeking means of improving the reliability and sensitivity of measurements of certain enzymes and isoenzymes released into the blood by injured heart tissue for determining the extent of initial heart-muscle damage after ischemia and assessing the results of therapeutic measures to limit or reverse that damage.



### How Heparin Prevents Blood Clotting

Thrombophlebitis occurs when a clot forms on the wall of an interior vein of the lower extremeties. If the clot, or thrombus, detaches itself from the wall, it may move up and partially or totally block the pulmonary artery and its branches which deliver blood to the lungs.

A detached thrombus is called an embolus and a very large one can cause a massive pulmonary embolism, which is responsible for an estimated 50,000 deaths a year in the United States. Pulmonary embolism frequently complicates surgery, pregnancy, and trauma. Pulmonary embolism in the U.S. causes annually some 5,000 to 10,000 deaths from operations which the patient would have otherwise survived. In addition, many survivors of thrombophlebitis develop chronic venous insufficiency.

Once a thrombus has definitely established itself on the vein wall, therapeutic measures which can be taken are the administration of anticoagulant or thrombolytic drugs, and physical measures such as heat application and rest. However, these measures do not always prevent the thrombus from becoming an embolism. This fact, coupled with the inadequacy of procedures now available to diagnose the established thrombus, make it clear that a preventive approach is more desirable.

Research over the past few years has clarified understanding of clot formation, stimulating the testing of preventive drugs.

One of the safest and most effective drugs found for preventing pulmonary embolism is heparin, a complex organic molecule produced commercially by the selective processing of pig intestines. Heparin was first identified as a naturally ocurring anticoagulant in 1915, but its unique anti-clotting action has been partially clarified only recently. Heparin had long been theorized to interfere in clot formation through activation of the blood protein, anti-thrombin.

The coagulation of blood occurs via two pathways in which activation of specific plasma proteins (known as clotting factors and identified by Roman numerals) initiates a "cascade" of reactions which leads to the formation of a fibrin clot, or thrombus.

Throughout the clotting cascade the factors are activating each other by "firing" serine protease "arrows" into their intended cascade factor "targets".

Theoretically, heparin interferes in the clotting cascade by increasing the activity of the molecule anti-thrombin to bind to Factor X and thrombin, inhibiting their clotting actions.

Research funded by NHLI and conducted by Dr. Robert D. Rosenberg, Chief of Thrombosis and Hemostasis at Beth Israel Hospital in Boston suggests that heparin-activated anti-thrombin binds not only the serine protease which activates Factor X and thrombin, but the serine proteases of factors further up the cascade. His findings are supported by the fact that heparin seemed to have almost instantaneous anti-clotting effect upon injection.

Dr. Rosenberg's research also proposes a clarification of heparin's potentiating effect upon anti-thrombin. Heparin carries a highly negative charge and it apparently binds to positively charged amino acids of the anti-thrombin. This binding activates the anti-thrombin's arginine, an amino acid with a high affinity for serine, which is the amino acid "tip" of the serine protease arrow.

The clinical implications of this finding are two-fold. First, more refined techniques may be developed to tailor an individual's dosage of heparin by a clearer understanding of how this drug blocks the clotting cascade. Secondly, the prophylactic or preventive use of low-dose heparin is gaining recognition as a useful therapy in the preventing pulmonary embolism in patients who are undergoing major surgery.

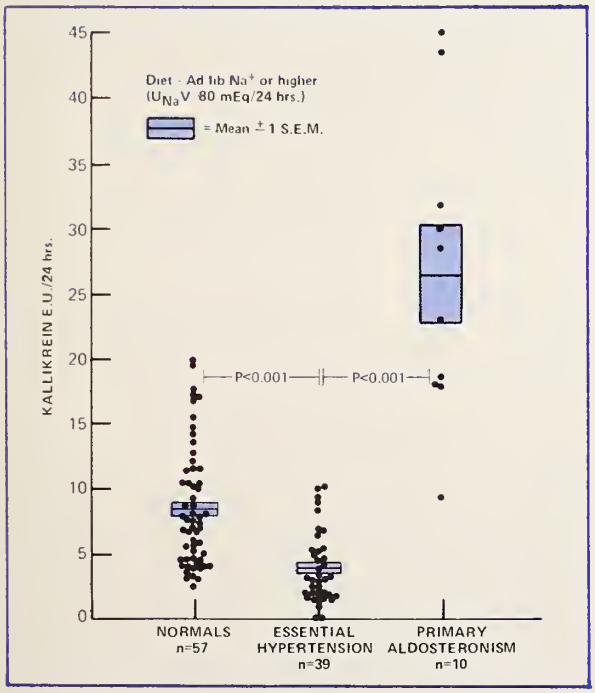
If Dr. Rosenberg's findings can be further elucidated, it may be possible to give a patient not a "standard" low dose, but an "ideal" dose during the critical periods which precede and follow major surgery.

## New Biochemical Approach To Hypertension

The regulation of blood pressure involves the complex interplay of many variables. These include the autonomic nervous system, which regulates many functions not under conscious control; blood electrolytes, notably sodium and potassium; various hormones, including aldosterone and others produced by the adrenal glands; and other "vasoactive" substances, which constrict or dilate blood vessels.

The recent development of fast, accurate, and sensitive techniques for measuring vasoactive substances and the products of their metabolism in blood, urine, and other body fluids promises to increase our understanding of their various contributions to blood-pressure control. These techniques may also shed new light on aberrations involved in the elevated blood pressure of hypertension.

The body's kallikrein-kinin system is thought to play important roles in the regulation of blood pressure and blood flow and also in the kidney's handling of salt and water balance. Kallikreins are enzymes in blood plasma and exocrine glands, such as kidney, pancreas and salivary




glands. The kallikreins act on inactive precursors called kininogens, present in plasma and various tissues, to produce active compounds called kinins. Several different kinins are generated, three of which are thought to be of physiological significance. All three share a common characteristic: they are powerful blood-vessel dilators.

The kallikrein-kinin system may also be involved in inflammatory reactions, chronic lung disorders, shock states, and the body's coagulation and clot-dissolving mechanisms. But NHLI studies have centered on their effect on blood pressure and flow.

In a sense, the prevailing blood pressure is the resultant of numerous forces—some contending against one another, others reinforcing one another, and still others working either side of the street depending on local circumstances.



As all known kinins are blood-vessel dilators, it would appear that the kallikrein-kinin system normally tends to oppose blood-pressure elevations resulting from activation of the sympathetic nervous system (which tends to constrict blood vessels); and from excessive secretion of aldosterone and other salt-retaining hormones from the adrenal glands. Evidence from NHLI studies does, in fact, support this basic hypothesis, though the relationships between the kallikrein-kinin system and other blood-pressure regulatory systems is seldom that simple.

The studies were carried out under the direction of Drs. H.R. Keiser and J.J. Pisano by Drs. H.S. Margolius, J.M. Vinci, D. Horwitz, Ronald G. Geller and co-workers.

The research thus far indicates that:

—Urinary excretion of kallikreins is abnormally low in subjects with essential hypertension. Studies among more than 600 healthy children and their mothers showed that urinary kallikrein levels tended to be similar in members of the same family and to be inversely proportional to blood pressure levels: that is, the higher the blood pressure, the lower the urinary kallikrein level and vice versa. Are elevated blood pressures, then, due in some measure to an impairment in the kallikrein-kinin system that normally acts to relax blood vessels?

—Urinary kallikrein excretion is abnormally high in patients with excessive aldosterone production, called primary aldosteronism, due to tumors of the adrenal glands. Aldosteronism may also raise blood pressure by promoting salt and fluid retention and expanding circulating blood volume. Urinary kallikrein determinations thus provide an effective means of distinguishing between this form of secondary hypertension and essential hypertension, whose causes remain obscure.

—Urinary kallikrein excretion rises and falls in

step with blood levels of aldosterone and other salt-retaining steroids. Kallikrein excretion is stimulated or depressed by the same stimuli as aldosterone secretion. For example, low-sodium or high potassium diets raise blood aldosterone and urinary kallikrein. Administration of the antihypertensive drug spironolactone (an aldosterone antagonist) also reduces kallikrein excretion. These findings suggest that the kallikrein-kinin system may be intimately involved in renal mechanisms governing fluid and electrolyte balance as well as those regulating blood pressure.



—In renal hypertension, which may develop as a result of an obstruction in an artery supplying a kidney, kallikrein levels are very low in urine collected from the blood-deprived kidney(s). Abnormally low urinary kallikrein levels, indicative of renal damage or ischemia, may thus be valuable in selecting patients whose renal hypertension is likely to be remedied by surgery.

—Plasma kallikrein is lower in women on oral contraceptives than in those not taking the pill. Data thus far are limited, but are interesting in view of the increased incidence of hypertension reported among pill users.

The role of the kallikrein-kinin system in blood pressure control remains to be defined, but that it plays an important role is no longer open to question.

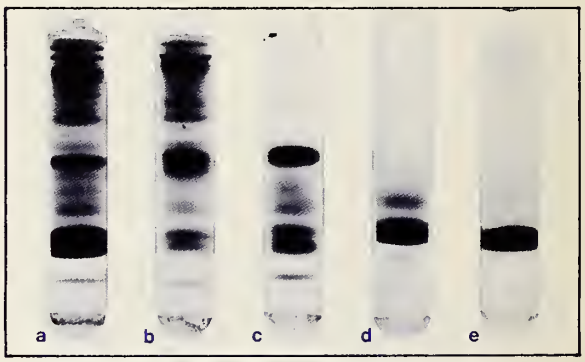
### Enzyme Isolation Advance Against Emphysema

A National Heart and Lung Institute grant-aided team of biochemists at the University of Georgia has isolated, in pure form, the enzyme antitrypsin (alpha-1-antitrypsin).

Their accomplishment—the availability of a purified antitrypsin—may be the key to defining the structure and function of this enzyme, and to understanding how severe, hereditary deficiencies of antitrypsin interact with environmental factors to produce a particularly severe kind of emphysema. Moreover, application of the biochemical isolation procedures to the more than 20 known variant forms of antitrypsin is likely to help delineate structural and functional differences among them, and to explain how enzyme deficiency states, involving one or more of these variant enzymes, can lead to disease.

Previous efforts to isolate antitrypsin were hampered by the difficulty of removing certain other blood protein contaminants, notably albumin and alpha-1-acid glycoprotein.

Drs. James Travis, David Johnson, and Ralph Pannel, of the Department of Biochemistry, University of Georgia, Athens, removed these contaminating substances through the use of affinity chromatography, taking advantage of the strong dye-binding capacity of albumin for its removal, and acidifying conditions during the final step of the procedure for separating alpha-1-acid glycoprotein from antitrypsin. The failure of a commonly used purity test to distinguish between alpha-1-glycoprotein and antitrypsin (which, in fact, is also a glycoprotein) lead the




Disc electrophoresis of plasma fractions (a) whole plasma. (b) Sepharose-blue dextran treated plasma. (c) G-75 fraction. (d) DEAE-cellulos, pH 8.8. (e) DEAE-cellulose, pH 6.5, pure alpha-l-proteinase inhibitor.

Georgia research team to conclude that previously "purified" antitrypsin preparations were actually badly contaminated.

In contrast, antitrypsin prepared according to their new procedure not only passes all tests of purity, but also has double the potency (trypsin inhibitory activity) of any other antitrypsin preparation.

The fact that at least some forms of antitrypsin deficiency can apparently interact with environmental factors (smoking, dust inhalation, and other forms of air pollution) to cause emphysema has led to the theory that one or more of the proteases (protein-dissolving enzymes normally inhibited by antitrypsin in the lung) are responsible for the lung damage in this kind of emphysema. These potentially destructive substances include trypsin and other proteolytic enzymes produced by the body, as well as proteases of plant and bacterial origin. Most of the available evidence implicates proteases that are released by white blood cells (leukocytes) and wandering "scavenger" cells (macrophages) as they do battle with bacterial and particulate lung pollutants.

It has been known for some time that antitrypsin deficiency is most pronounced—and the risk

of early, severe emphysema highest—in those persons who are *homozygous* for the trait; that is who have inherited it from both parents. And it is assumed, but not yet proven, that the risk may still be substantial in *heterozygotes*, those who inherit the deficiency from only one parent, a group which may comprise some 5 percent of the population. Obviously, if the progression of lung disease can be prevented in these antitrypsin-deficient persons by avoidance of smoking and exposure to lung irritants, then a means of identifying these persons would have value.

However, multiple population studies to determine the significance of intermediate (and supposedly heterozygotic) enzyme-deficiency states produced contradictory results, and were further negated by the recent discovery of more than 20 variant forms (phenotypes) of antitrypsin, including heterozygous and homozygous forms of each.

Thus emerged the need to consider qualitative as well as quantitative differences in antitrypsin levels, and to correlate these differences with the occurrence of pulmonary disease. Several such studies are underway in different parts of the country.

Identified collectively as the protease inhibitor (pi) system, the antitrypsins discovered thus far have been detected by *electrophoresis*, in which the enzymes migrate various distances in an electric field according to their electrical charge. The enzymes have been classified and labeled alphabetically according to their relative mobilities in the electrophoresis test system. Thus, the most common phenotype, homozygous type M (designated pi MM), possesses intermediate mobility, whereas the rare variant pi ZZ migrates very slowly and travels but a short distance during the course of the test. Electrophoretic mobility of the heterozygous phenotype pi MZ is intermediate between pi MM and pi ZZ.

Certainly the availability of purified antitrypsin will greatly enhance further attempts to define protease inhibition and other functions of antitrypsin, including studies of its biosynthesis, transport, sites and mechanisms of action, and breakdown within the body. The precise characterization of antitrypsin phenotypes should enable more meaningful comparisons of these variant enzymes, not only in terms of identifying those phenotypes predisposing to disease, but possibly revealing therapeutic approaches via 1) stimulating increased production or release from storage sites of antitrypsin in the body; 2) correcting the deficiency by injections of antitrypsin, or; 3) laboratory synthesis of antitrypsin or of active portions of the molecule capable of counteracting the damaging effects of a particular protease.

### Is High Altitude Pulmonary Hypertension Genetic?

The stresses of oxygen deficiency, which we begin to feel at altitudes above 6,000 feet, continue to pose challenging research problems. Central among these is the tendency of the smaller branches of the pulmonary artery to constrict as the oxygen tension drops in the lung airways. It is not known exactly why or how this happens, but the tendency is quite general in humans and some other mammals.



To pump enough blood through narrowed lung vessels, the heart must generate a higher pressure. This results in pulmonary hypertension as well as some enlargement of the right ventricle, which pumps to the lungs. Right-sided congestive heart failure often results when the altitude stress is extreme or the heart or lungs are already compromised by disease. Since many heart and lung ailments involve hypoxia, pulmonary hypertension and congestive heart failure, knowledge of the biological mechanisms underlying high altitude sickness would contribute to an understanding of many cardiorespiratory problems.

Cattlemen know as "brisket disease" a like syndrome of pulmonary hypertension and heart failure in herds on high mountain pastures. In fact, high mountain disease is an important economic problem in many areas, with up to 40% of the cattle in some herds developing severe pulmonary hypertension and heart failure when moved from lowland to pastures above 10,000 feet.

But some cattle in a herd—said to be "resistant"—develop only mild pulmonary hypertension and thrive at the same altitudes where others—termed "susceptibles"—succumb to severe pulmonary arterial pressures and congestive heart failure. This characteristic of brisket disease has engaged the attention of NHLI grantees in Denver and Fort Collins, Colorado, for it also holds true of the human high altitude sickness.

By the early 1960's Drs. Donald H. Will and Archibald F. Alexander, veterinarians at Colorado State University, had theorized that susceptibility and resistance to hypoxic pulmonary hypertension are genetically inherited traits, at

least in cattle. In 1965 they began to single out resistant and susceptible animals from high mountain herds for breeding experiments necessary to test this theory. Susceptibles with obvious heart failure (swollen brisket) were brought down to Fort Collins and allowed to recover for mating to other susceptibles, and the development of two separate Hereford lines was begun. Very early in the Fort Collins work, the implications of the theory for human disease brought the collaboration of Dr. Robert F. Grover and his associates from the University of Colorado School of Medicine in Denver.



Dr. Grover had long been interested in the great differences in human cardiopulmonary susceptibility between populations living in the Peruvian Andes at high altitudes for as long as 9,000 years and newer high altitude communities of European stock, such as Leadville, Colorado, where only two generations of families had lived from birth at 10,200 feet, and where pulmonary hypertension is a relatively common problem. The high altitude tolerance of the Andean Indians is legendary, with people dwelling in communities as high as 17,500 feet.

By 1975, the Fort Collins-Denver team had clearly demonstrated that susceptibility and resistance in cattle to hypoxic pulmonary hypertension are genetically inherited traits, which had been transmitted through three generations of the two herds of cattle. The descendants of the cattle that had recovered from brisket disease invariably developed severe pulmonary hypertension and congestive heart failure when moved to 10,000 feet, while the descendants of the resistant individuals developed only mild, innocuous pulmonary pressure elevations. The cattle now serve as a model for inquiry into the mechanisms by which hypoxia causes the vessels to constrict, and the Colorado investigators are exploring differences in vessel wall musculature and innervation which might throw light on this puzzling phenomenon.

"The genetic factor in high altitude pulmonary hypertension had not previously been shown," Dr. Grover explains. "While the studies were conducted in cattle, the general principles derived may well apply to other species, including man."

### The Structure of Apoproteins

Research interest in mechanisms of fat (lipid) treatment in the body has intensified in recent years, partly because certain lipid transport disorders characterized by abnormal elevations of blood lipids have become recognized as a major risk factor in the development of atherosclerosis and its manifestations such as heart attacks and strokes. But recent research advances have also opened the way for further exploitation of this complex area of study.

Several of these key advances pertain to certain blood proteins called *apoproteins,* natural "detergents" actually, that combine with lipids—cholesterol, triglyceride, and phospholipid—to render them soluble in the watery transport medium that is blood.

These fat-protein complexes, called *lipoproteins,* are produced mainly by the liver and intestine. The lipoproteins are divided into four classes on the basis of density. The lightest are the *chylomicrons,* large particles of dietary triglyceride containing only about one percent by weight of protein. Next are the *very-low-density (VLDL or pre-beta) lipoproteins,* which contain 2-15 percent protein. *Low-density (LDL or beta) lipoproteins* contain 20-25 percent protein, and *high-density (HDL or alpha) lipoproteins* contain 45-55 percent protein.

Each lipoprotein transports a mixture of lipid, so that triglyceride, cholesterol, or phospholipid may travel in combination with lipoproteins of any weight class. However, chylomicrons and VLDL transport most of the triglycerides of plasma, LDL most of the cholesterol, and HDL most of the phospholipid. While phospholipid is the least abundant of the three major lipid fractions, it apparently has an essential stabilizing effect: without it, neither cholesterol nor triglyceride will bind to apoproteins to form lipoprotein molecules.

High-density lipoproteins and the lipids they carry do not appear to be atherogenic; and a robust HDL fraction may actually confer some protection against the development of atherosclerosis. However, excessive blood levels of VLDL result in elevated blood triglycerides and excessive blood levels of LDL result in elevated

blood cholesterol; both are strongly associated with premature atherosclerosis and coronary heart disease. During recent years, studies have shown that blood-lipid abnormalities should be considered in terms of lipoprotein abnormalities, termed *hyperlipoproteinemias.*

Recent research has revealed that at least six different apoproteins are components of the lipoproteins of human plasma. As is the case with lipid, each lipoprotein class may have several apoprotein constituents, some of them present in other lipoprotein classes.

Dr. Richard Jackson (r.), of Baylor College of Medicine, discusses a model of plasma lipoproteins with Dr. Antonio M. Gotto, Jr.

The isolation and purification of the various apoproteins of human lipoproteins was a step toward 1) identifying and classifying genetic factors operative in lipoprotein synthesis and in hereditary forms of hyperlipoproteinemia; 2) shedding new light on lipoprotein structure and the differing affinities of the various lipoproteins for the lipids of human plasma; 3) clarifying the mode of action of lipid-lowering diets and drugs; and 4) bringing to light other functions of apoproteins, other than conferring solubility on the lipids being transported, that may be important in lipid metabolism.

Since 1971, when National Heart and Lung Institute scientists determined the amino-acid sequence of the first of these apoproteins, studies here and elsewhere have revealed most of the chain structure of four other apoproteins.

Until quite recently, however, the structural information yielded no clues as to how apoproteins combine with lipids. Now, work by Dr. Antonio M. Gotto, Jr., and colleagues at the Baylor College of Medicine and The Methodist Hospital, Houston, provide a plausible explanation of how some apoproteins combine with phospholipid.

Working with polypeptide fragments of dismembered apoprotein chains, Dr. Gotto and coworkers found that only some of the fragments combined with phospholipid but, in so doing, assumed a more pronounced helical structure as compared to those fragments displaying no affinity for phospholipid. These preliminary findings led to a re-examination of the known amino-acid sequences of apoproteins to see whether any of the structural features could account for their observations.

What emerged was a new view of the apoprotein molecule as a unique, two-faced or *amphipathic* helical structure with a large non-polar face that binds with the fatty acid chains of phospholipids, and a polar face that combines with water-soluble portions of phospholipid. Computer searches of existing structural data enabled the construction of molecular models of three different apoproteins, in which the arrangement of amphipathic regions and charged groups were consistent with the new theory. Lending further support to their views are the results of phospholipid-binding studies with three model peptides synthesized in the laboratory. Each of the model peptides were amphipathic and contained similarly arranged pairs of oppositely charged amino acids, but one differed from the others in that its non-polar face was considerably more *hydrophobic,* or water-repellant. That phospholipid combined only with this peptide indicates the importance of hydrophobicity of the non-polar face of the helix in the binding of phospholipid.

Dr. Gotto and the research team he heads were aided by grants and contracts from the National Heart and Lung Institute, the John A. Hartford Foundation, Inc., and the American Heart Association, Texas Affiliate.

The newest component of NIH, the National Institute on Aging was in its first year of operation in Fiscal Year 1976. The Institute conducts and supports biomedical, social and behavioral research and training related to the aging process and to the special health problems of older people. Research by NIA scientists and grantees is providing the basic knowledge that will help promote a healthy and productive later life for all people.

### Reasoning Ability and Age

The ability to reason and to solve problems logically is prized by young and old alike. Gerontologists are interested in whether or not this ability to reason changes over the years.

Dr. David Ahrenberg and his associates at the NIH's Gerontology Research Center, have shown there is a decline in performance by older men. However, this decline was seen only for those subjects who were over 70 years of age when first tested. The men tested are participants in the Baltimore Longitudinal Study. They range in age from 24 to 87 years, most have a bachelor's or an advanced degree, and all live in the community and visit the Center periodically to undergo a battery of physiological, medical, and behavioral measures of age changes.

Initially, 300 men undertook three logic problem-solving tasks to measure their reasoning performance. Comparisons of performance of the young and old men showed that the proportion of men who successfully solved each problem decreased with age.

Some six years later, 224 men out of the original group were retested at the Baltimore Center. This gave the researchers measures on the same men at two different ages. The results showed a decline in problem-solving ability only for those men who were past age 70 years when first tested. Most of the declines noted resulted from non-informative inputs; that is, the men made choices during the tests or asked questions that could not provide any new information to help them solve the problem at hand. The high incidence of repeated responses among the old was somewhat surprising since memory demands were minimized by having each subject write down his actual input and its outcome (result) thus making the entire record of test events readily available.

This light box was used for studies showing a decline in problem solving performance by older volunteers.

The investigators conclude that the ability to solve problems does decline in late life even in a group of highly educated people. However, many of the older subjects, especially those first tested while in their sixties showed no decline in performance on retesting. This suggests that at least some older men maintain high reasoning ability even into advanced ages.

### Mental Change with Age

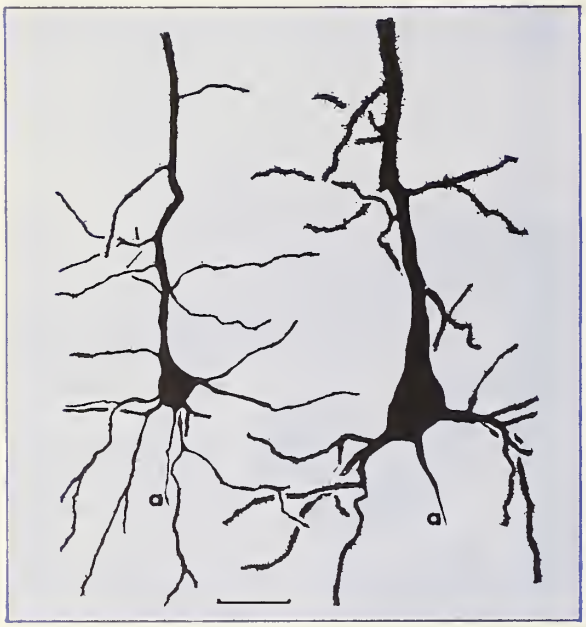
One of the most distressing aspects of aging is the mental deterioration of the elderly called "senility." Individual cases of mental senility are probably due to mixtures, in varying proportions, of diseases occurring in some people, and of deteriorative changes occurring in everyone with increasing age and classified as aging processes.

Recent studies conducted by Institute-supported scientists provided evidence that in rats there is a progressive loss of the ability to remember newly acquired information that occurs throughout the entire adult life and that this may be asystematic. Dr. James L. McGaugh at the University of California at Irvine found a striking decrease in memory power in rats from early adulthood to the middle stage of life and an additional decrease when rats are entering old age. These results were found in two quite different kinds of memory tests. Although increased forgetfulness with advancing age has long been seen in some human beings, the evidence heretofore has been based on casual observation or on controversial findings, with some regarding forgetfulness as a consequence of pathology or extreme old age. Dr. McGaugh's unusually careful observation on healthy animals of various ages makes a strong case that increasing forgetfulness is a regular phenomenon across adult life in normal, healthy mammals. More importantly, Dr. McGaugh's neurochemical findings strongly suggest the precise nature and locus of brain activities which decline with age proportionally to the decline in memory capacities. If his findings hold up for higher mammals than rats and if the memory-

neurochemical correlations are not just an unusual coincidence, experiments in improving memory by biochemical therapy might one day be possible.

Another study of the aging rat brain by Dr. Martin Feldman of the Boston University School of Medicine has shown a striking anatomical change with age. This change consists of a considerable reduction in the number of connections (synaptic connections) between the nerve cells that form important portions of the brain in higher animals (portions of the cortex).





Portions of pyramidal neurons from layer V of the visual cortex from a young rat (r.) and an old (l.) rat. Aged dendritic branches show a loss of the small spine-like protrusions. These spines are the sites where nerve impulses from other neurons are received. a, axon. Calibration line, 25 um.



The impairment of learning in the rat seems similar to the changes that occur in human beings. The anatomical studies reported above would be very difficult to conduct in humans because human brain tissue can ordinarily be obtained only from patients who have died of some disease which could have caused some brain changes. In addition, brains obtained after natural deaths ordinarily have undergone enough post-mortem changes to make detailed microscopic studies very difficult to interpret.

Both of these findings suggest that by using the rat as an experimental animal, something can be learned about human senility.

## High Blood Pressure and Intellectual Performance

Another NIA grantee, Dr. Merrill F. Elias, of Syracuse University, has been examining the effects of sustained high blood pressure on the behavioral performance of young (18-31), middle-aged (32-45), and older adults (46-59), in collaboration with various colleagues at the Veterans Administration Hospital, Syracuse, New York. In a section of the project directed by Ms. Kathy Light, testing requiring selection of the correct response from as many as eight alternatives was performed with young and middle-aged men and women to determine the extent to which rapid decision making and responding is impaired by the effects of aging and high blood pressure.

Results indicated that the speed with which people make responses involving complex discriminations declines with age, but that many people with high blood pressure perform even more poorly than healthy subjects. Although not all of the patients with elevated blood pressure reacted more slowly than controls of the same age, those whose blood pressure had been previously controlled by medication showed greater incidence of slowing. The type of hypertensive disorder each patient has seems to indicate whether medication is related to improvement or deficit in performance.

Further pilot studies are being undertaken in collaboration with Upstate Medical Center in order to determine the extent to which decision making under time pressure is affected by plasma renin levels and cardiovascular and cerebrovascular complications associated with hypertension and aging.

## Self Control of Blood Pressure

High blood pressure (hypertension) is a serious and life threatening condition that affects at least 23 million Americans, claims over 20,000 lives each year, and contributes to hundreds of thousands of deaths annually from heart attacks and strokes. The National Institute on Aging is looking into ways of treating this condition in the older adult.

Drs. Bernard T. Engel and Donald A. Kristt at the Gerontology Research Center are using operant conditioning techniques (training by reinforcing successful behavior) to train patients with high blood pressure to voluntarily control their own blood pressures.

Five patients, men and women 46-70 years of age, have learned to increase and decrease their

blood pressures in the laboratory reliably. After the laboratory training they continued training in their own homes. All of the patients were able to exercise control throughout a 3-month follow-up period.

Subjects for the study were selected from the Baltimore City Hospitals hypertension clinic. Their training was divided into three phases. First, five weeks before hospitalization, patients learned to record their own blood pressures at home and mail the results to the Center. The patients were then admitted to the Gerontology Research Ward of the Baltimore City Hospitals.

The second phase consisted of laboratory training. The patient lay in bed in a quiet room. In front of the patient was a display of red, yellow, and green lights, much like a traffic light. When the red light was on, the patient tried to lower systolic blood pressure (the higher pressure on a blood pressure reading). The yellow light was on while the patient responded correctly (lowered blood pressure). This light is the patient's "feedback" telling him he is doing the right thing and reinforcing his successful behavior. Another reinforcement tool is a meter which gives the patient an accumulated numerical score of performance; each successful response advances the meter by two points. The final light, green, is used to tell the patient to raise blood pressure. Both raising and lowering are used to demonstrate the ability to control blood pressure, and to enable the patient to develop a sense of the difference between rising and falling blood pressure.

The final phase involved training the patients to practice blood pressure control at home. They were asked to practice this training several times a day and to record their performance over a 3-month follow-up period.

This study confirms and extends the work done previously in other laboratories by showing that the patient can maintain and continue the skills in his own home for at least 3 months. These findings suggest that patients may control systolic blood pressure by regulating resistance by the arterioles to the flow of blood from the arteries to the veins, since the blood pressure changes occurred in the absence of any changes in heart rate.

## Immunology of Aging

The ability of the body to protect itself against disease (immune function) decreases with age at the same time that various protective systems begin to malfunction. The observation made some 45 years ago that the concentration of natural antibodies, the most important defenses against foreign invaders such as germs, declines with age is probably the earliest scientific evidence we have of this system. Recent discoveries have shown the importance of this loss, at the same time that the possibility has arisen that this can be improved by medical treatment.

At the University of California, Los Angeles, Dr. Roy L. Walford found that by restricting the number of calories or the protein content of the diet of laboratory rodents he was able to prolong their lifespan by 15 to 40 percent, to lower the incidence and growth of spontaneous and transplanted tumors, and to increase resistance to some viral infections.



The mice were first tested at 3 to 4 months with a variety of immunologic measuring devices. The immune responses of the restricted mice were less than those of the controls, but by one year of age, this was reversed. The regulated animals possessed an immune system which remained or behaved younger longer than animals on a nonrestricted diet. Dr. Walford is also examining various protein dietary combinations to study their effects. Severe restriction of protein, he found, had an undesirable effect.

## Removal of Old Cells

An important, but little understood, biological problem deals with the mechanisms used by scavenger cells (macrophages) of the immune system to recognize and remove deteriorated cells from the body.

At the Laboratory of Cellular and Comparative Physiology, Gerontology Research Center, Dr. Marguerite M.B. Kay has chosen the human red blood cell to study this problem since macrophages routinely ingest and destroy red blood cells at the end of their 120-day useful life span in the circulation.

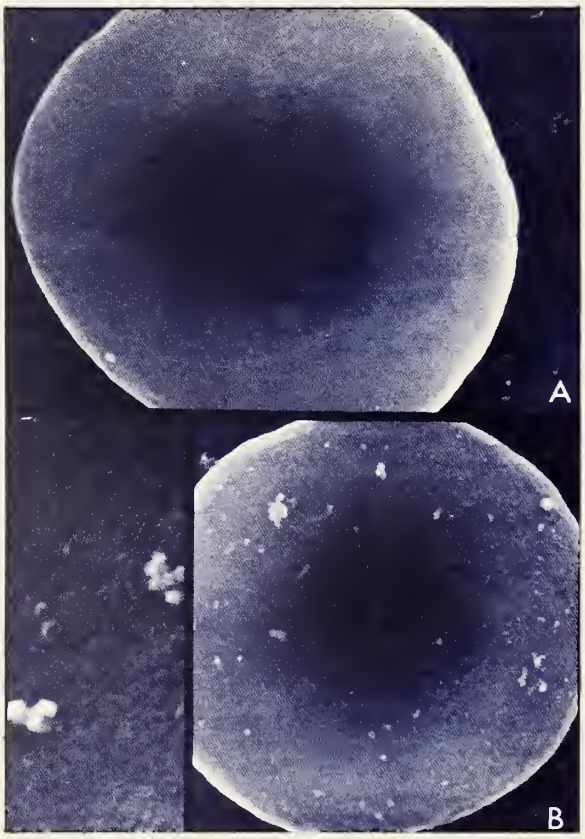
In some manner, macrophages can distinguish between "self" and "non-self"—that is, they remove foreign cells while leaving the body's cells alone. However, they also destroy old "self" cells so they must be able to distinguish between red blood cells that are still doing their job in the body and those that are at the end of their usefulness.

Research done in other laboratories suggests that the macrophages recognize certain signs, cellular alterations, which help them single out deteriorated cells. These signs tell the scavenger cells which red blood cells should be destroyed,

without endangering functional cells.

The NIA investigators used experimental methods different from those employed by other laboratories to study macrophage recognition of old red blood cells. They separated freshly drawn young and old human red blood cells by weight which preserved them in a near natural state. In the past, such studies used cells aged in tissue culture. The cultures used to age these cells can damage or change the cells under investigation.






Macrophages may recognize old red blood cells (RBCs) to be removed by a buildup of Immunoglobulin G on cell surfaces. Scanning electron microscopy shows (a) low magnification of young RBC demonstrating smooth surface. (b) low magnification of old RBC indicating 'spots' on surface. (insert) high magnification indicates 'spots' are accumulated IgG on surface of old cells.

Previous research, using cells aged in tissue culture, suggested that the sign read by the macrophages could be a reduced negative electric charge on the surface of old cells. However, this theory depends heavily on the macrophages having sites capable of recognizing such charges. To date, there is no proof that such sites exist so the validity of the negative charge theory remains unproven.

On the other hand, early evidence from the Gerontology Research Center investigations indicates that macrophages differentiate between young and old cells by recognizing the accumulation of immunoglobulin G on the surface of the old red blood cells. Immunoglobulin G is a protein in human blood involved in immune responses. The investigators used scanning electron microscopy to detect immunoglobulin G on both young and old red blood cells. This revealed that the young cells had only trace amounts of immunoglobulin while the old cells had a definite accumulation.

These findings point to a gradual build up of immunoglobulin G as red blood cells age in the circulation. When a critical level is reached, the macrophages no longer recognize the cells as "self," that is, as part of the normal circulation. They then ingest and destroy these cells.

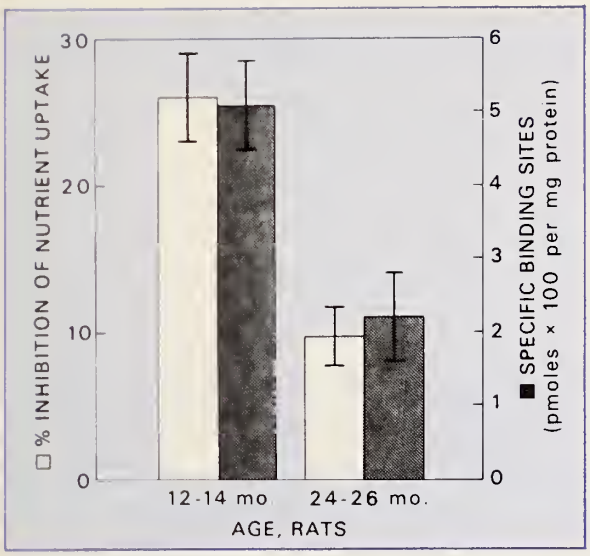
### Hormonal Responses and Age

One of the principal ways the body integrates its growth and metabolic functions is through the complex hormonal system. Many defects in hormonal activities have been demonstrated with aging. Among these is the decreased response of target tissues to hormonal stimuli.

The first biological event needed to elicit most hormonal responses is the binding (attachment) of hormones to specific sites (receptors) in the cells to be stimulated. NIA Gerontology Research Center scientists are studying old rats (24-26 months) and mature animals (12-14 months) to evaluate the responses elicited and the sites available for hormone binding in both young and old animals.

An examination of splenic leukocytes (white blood cells in the spleen) showed that there was a definite reduction in at least one hormonal response of old cells. In this case, the response was steroid hormone inhibition of nutrient uptake by the cells. The cells from old animals had less ability to bind hormones, and the number of intracellular sites available for hormonal binding was significantly less than was seen in the cells from the young animals.

Previous studies at the Center and elsewhere have shown that the amount of hormone which binds to specific sites greatly influences the hormonal response elicited. In this study, the decrease in the old cells' responsiveness was essentially equal to the decreased number of steroid hormone binding sites available. In addition, when the binding sites in old cells were artificially blocked with other hormones, no




The decreased response of old animals to hormonal stimuli is due in part to fewer binding sites on cells to be stimulated (dark columns). The desired response, steroid hormone inhibition of nutrient uptake, is obviously less in older rats (light column).

response could be induced.

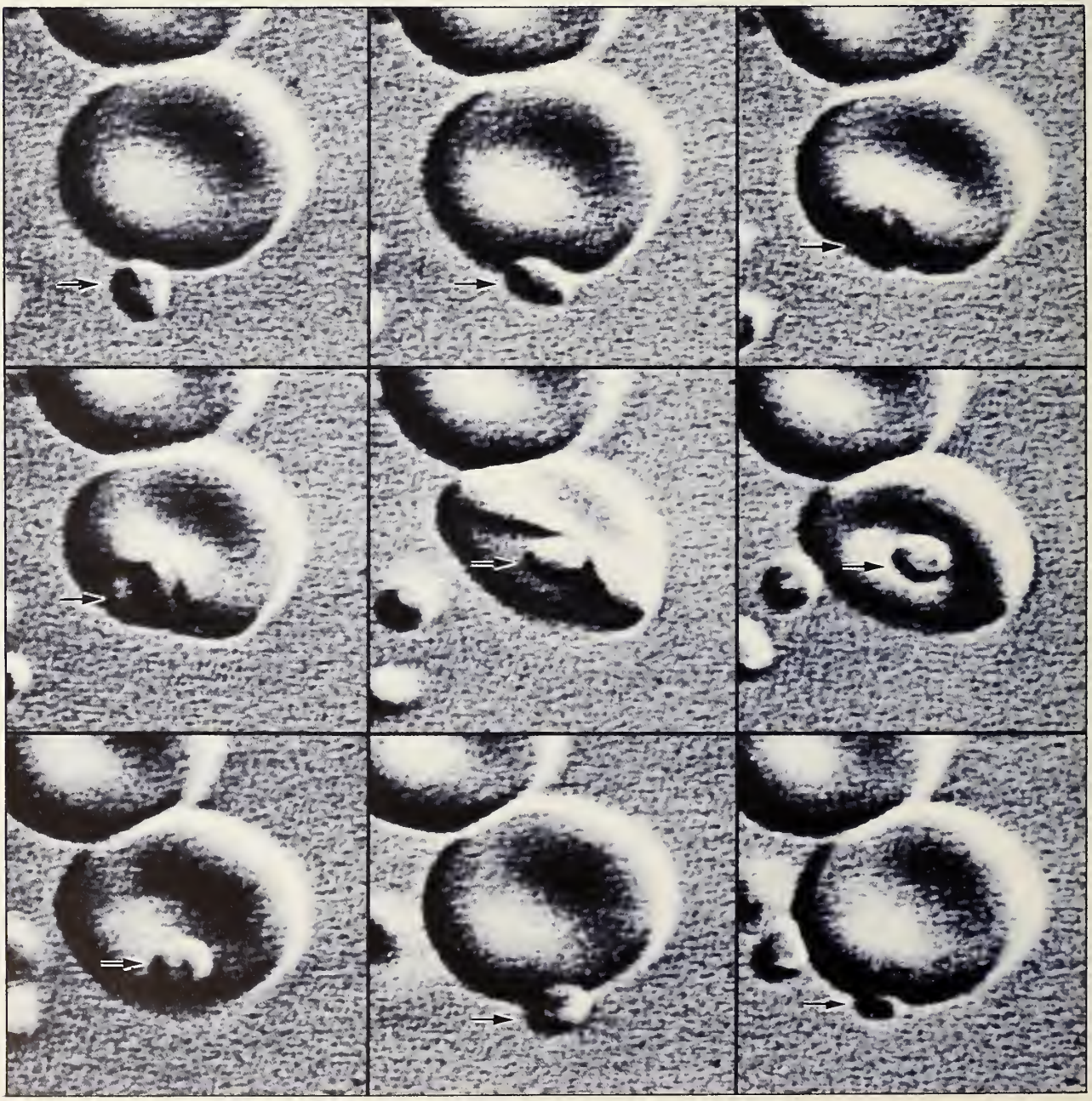
The results point to a probable link between reduced hormone binding and responsiveness, although further research is necessary to verify that changes in hormone binding are the general cause of altered hormonal responsiveness in the old.

Investigations are now underway to explain the mechanisms responsible for age-related changes in hormone binding ability. The researchers are investigating hormone binding changes in target cells from tissues that do not proliferate or change appreciably during the life span, for example, fat cells, neurons, and muscle cells.

Such studies may eventually answer a crucial question affecting the general health of older people: How do age-related changes in hormone responses occur, and can they be halted, prevented, or reversed by chemical manipulations?

This inverted microscope sequence shows (from upper left to lower right) the invasion of a red blood cell by a malaria parasite (arrow). Following attachment of the parasite to the red blood cell, there is a marked distortion of the red blood cell followed by the relatively slow invasion of the cell by the parasite.





## Hepatitis Studies

Although the National Institute of Allergy and Infectious Diseases (NIAID) conducts and supports research on all organisms causing human infections, it is no accident that the bulk of its effort is devoted to obtaining a better understanding of ways to control and combat disease-causing viruses. Among acute conditions, virus infections in this country account for most patient visits to a physician, are responsible for most days lost from work or school, and are a special threat to the very young and the very old.

Viruses are true parasites, unable to reproduce and maintain their existence except in cells of a susceptible host. This intimate relationship between viruses and living cells has made it extremely difficult to devise medications which will harm one and not the other. However, the past century has witnessed a growing list of vaccines developed by scientists to immunize man against various viral invaders; and during the past year, NIH investigators have reported exciting progress toward the development of a vaccine for yet another worldwide viral disease problem: hepatitis B, or serum hepatitis. Transmission of this form of viral hepatitis has been most frequently associated with the use of contaminated blood or blood products and a vaccine is needed, particularly for use in high-risk groups.

Although the virus causing hepatitis type B has now been identified as the so-called Dane particle, it has not yet been possible to grow the organism in tissue culture—ordinarily the first step in vaccine development. Therefore, Drs. Robert Purcell and John Gerin, NIAID, used as starting material for their novel, prototype vaccine, plasma or sera from human chronic carriers of the hepatitis B surface antigen, formerly known as the Australia antigen. This antigen (a substance inducing the formation of blood proteins known as antibodies) is shared by the Dane particle and by much smaller spherical particles found in abundance in the blood of hepatitis B carriers.

Using high speed centrifuges, the scientists first removed from the starting material any infectious hepatitis virus. The remaining, small, non-infectious particles were themselves then purified by centrifugation and inactivated by treatment with the chemical, formalin.

The vaccine preparation was next tested for potency in guinea pigs and was found capable of inducing the formation of specific antibody. When the vaccine was tested in chimpanzees, it was found safe and effective in protecting the animals against hepatitis B.

This work, most of which was carried out under contract and in collaboration with other Federal scientists (Bureau of Biologics, of the Food and Drug Administration and the Center for Disease Control), has provided a strong scientific base for expanded studies of hepatitis B immunization now underway at NIH and elsewhere.

Other viral hepatitis studies last year highlighted a new area of concern. NIAID and NIH Clinical Center scientists have obtained convincing evidence that many cases of post-transfusion hepatitis are not due to hepatitis B or hepatitis A—the two hepatitis agents most frequently recognized—but may be caused by other yet-to-be-identified viruses.



During the past few years, operators of blood banks have found that by pre-testing donor blood for the presence of the hepatitis B surface antigen and eliminating positive and/or commercial donors, the incidence of post-transfusion hepatitis can be substantially reduced. However, some cases still occur and approximately half of these cannot be related, even by the most sensitive test methods, to the hepatitis B virus. It had been assumed that most of these non-B, post-transfusion cases were probably hepatitis A, although the incubation period often exceeded that generally accepted for this form of the disease.

When NIAID scientists in 1973 showed that the technique of immune electronmicroscopy was a useful means for serologically documenting hepatitis A infection, it became possible to test this assumption. Last year, Drs. Stephen M. Feinstone, Albert Z. Kapikian, and Robert H. Purcell, NIAID, and Drs. Harvey J. Alter and Paul V. Holland, NIH Clinical Center, used immune electronmicroscopy to examine paired sera obtained from 23 selected cardiac surgery patients prior to and 6 months after receiving blood transfusions. Although all of these patients had post-transfusion hepatitis, there was no evidence of hepatitis B infection in their sera. There was no antibody response to the hepatitis A antigen, either, which would have constituted evidence of hepatitis A infection. Antibody responses to other viruses—such as herpes and cytomegalovirus—known to affect the liver were also studied, but there were no changes that could relate these agents to the development of post-transfusion hepatitis.

Other hepatitis studies focused on the degree of hepatitis risk faced by various groups coming in

contact with persons positive for hepatitis B surface antigen.

To assess the risk of patient contacts of hepatitis B positive health workers, Dr. Alter of the CC Blood Bank, and co-workers followed, for 6 to 9 months, 228 Clinical Center patients who had been served by hepatitis B antigen positive health workers. Controls in the study were 167 identically followed Clinical Center patients who had not been exposed to a hepatitis B antigen positive health worker. No patient in either group acquired clinical hepatitis or became positive for hepaitits B surface antigen.



In contrast, family members exposed to acute leukemia patients positive for the hepatitis B antigen were at significant risk of developing hepatitis. When these persons were followed for 18 months, it was found that 5 of 24 mothers of leukemia patients with hepatitis B surface antigen developed acute, type B hepatitis and that 4 of 40 siblings developed antibody to the hepatitis B antigen. In contrast, none of 161 relatives of hepatitis B antigen negative leukemia patients developed hepatitis or a serologic response.

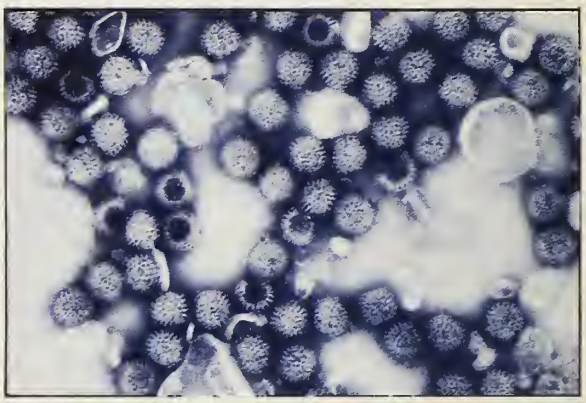
The investigators—Susan C. Steinberg, Clinical Center Nursing Department, Dr. Alter, and Dr. Brigid C. Leventhal, National Cancer Institute—found that family members who regularly shared drinking glasses and food had the highest frequency of hepatitis and seroconversion. Since this suggests oral rather than blood-borne transmission of infection, the investigators recommend that family members be counseled concerning ways to minimize direct contact with antigen-containing body fluids, such as saliva, while maintaining warm physical and emotional support of the leukemia patient.

### Virus Causing Infant Diarrhea Identified

In other virus research during the past year, an NIAID scientist and his co-workers have identified, for the first time in the U.S., the virus causing a severe infant diarrhea. They have also established a serologic relationship between this agent and one causing a similar disease in newborn calves. The latter observation has resulted in the development of a simple diagnostic test and has led the investigators to speculate that it may be possible to use the calf virus as the basis for a human vaccine.

Infant diarrhea has long been one of the most common causes of childhood illness and, in many parts of the world, is a leading cause of infant death. It has been estimated that probably no more than one-eighth of the world's infants and children are free from appreciable risk of lethal diarrhea. Although some diarrhea cases can be attributed to bacteria or to parasites, an even more important cause seems to be the reovirus-like agent recently investigated by NIAID's Dr. Albert Z. Kapikian and his colleagues.

Using both immune and conventional electron-microscopy, the scientists observed the new agent in the stools of young children hospitalized in Washington, D. C. and established it as



Reovirus-like particles identified world-wide, as cause of severe infant diarrhea.

the cause of their severe diarrhea. Their findings confirmed earlier observations on the occurrence of a virus-like particle in the feces of Australian infants with diarrhea. The virus was later shown to be identical with similar agents observed in the stools of children with diarrhea in England, Canada, and elsewhere.

In a follow-up to these studies, the NIH investigators observed that a large proportion of children infected with the human agent developed serologic evidence of infection with a calf diarrhea agent as well. The scientists also found that they could use the calf diarrhea virus as the antigen in a new complement fixation test for diagnosing acute gastroenteritis in children. The ready availability of this culture-grown Nebraska calf diarrhea virus and its ability to serve as a "substitute" for the human agent, which cannot yet be cultured in quantity, should greatly facilitate epidemiologic studies of infant diarrhea.

In addition, evidence of the close relationship between the two agents raises the possibility of immunization against human disease with the use of calf virus. An orally administered attenuated calf virus vaccine is already in use in veterinary medicine.

In a serologic survey of residents of a children's

institution, antibody to the reovirus-like agent was found in the sera of over 80% of individuals by 36 months of age. Thus, members of this young age group, who are at high risk of developing severe diarrheal disease, would be prime candidates for immunoprophylaxis if a safe, effective vaccine is developed for the new acute gastroenteritis virus.

### Cellular Immunity Stops Herpes Virus

Not all virus research, of course, is directed toward development of vaccines. Scientists hope that, by obtaining a better understanding of how viruses establish themselves in cells and how the body reacts to their presence, it may be possible to develop various means for preventing viral infections or even for treating them.

Of special concern are the herpes simplex viruses. Members of this virus family have an unusual facility for establishing latent infections. Under these circumstances, the virus enters the host's cells, often without repercussions, then remains quiescent until the infection is "reactivated." Herpes disease may take the form of "fever blisters" on the lips or around the nose, "canker sores" on the gums and inside the cheeks, or, more seriously, infections of the genital tract or eye.

The body's immune response to a foreign substance, or antigen, is dependent upon the interaction of antigens, antibodies, and specialized cells. When an antigen induces white blood cells (lymphocytes) to form antibodies, *humoral immunity* is said to be involved; when lymphocytes react directly with the antigen, *cellular immunity* is called into play.

Last year NIH scientists found that at least one aspect of cellular immunity—the release of the substance, interferon—is required to stop the cell-to-cell spread of herpes simplex virus (HSV). Results of previous studies have suggested that cellular immunity is involved, but the precise way in which cell-mediated immunity protects against HSV has not been clear.

Dr. Donald L. Lodmell, NIAID, and Dr. Abner L. Notkins, National Institute of Dental Research, set up experiments to see 1) whether sensitized lymphocytes stimulated by a specific antigen were more effective than unsensitized lymphocytes in inhibiting HSV replication, and 2) whether chemical mediators released by sensitized lymphocytes could inhibit viral replication by acting on as yet uninfected cells.

The scientists found that both questions could be answered in the affirmative. Even a few sensitized and immunologically stimulated lymphocytes added to cultures of cells infected with HSV were able to inhibit more than 90% of viral replication. Moreover, the immunologically stimulated, sensitized cells released into the culture fluid a substance, which, by itself, could inhibit viral replication. This substance was found to have the characteristics of the chemical mediator, interferon, which is produced naturally in the body in response to invasion by viruses or certain other intracellular parasites.

The investigators believe that results of their study support and expand the concept that the body's immunological defense against HSV consists of two phases: In the first phase, antiviral antibody, complement (a system of blood proteins essential for the antigen-antibody reaction), and immune cells react with virus or virus-infected cells. In the second phase, nonspecific cell toxicity produced by inflammatory cells and the action of immunologically-induced interferon finally stop spread of HSV. Because the virus spreads so rapidly from cell to adjacent cell, the first phase has little effect on halting the infection. Instead, it is the second phase that stops HSV spread, mainly by generating interferon.

### New Papovavirus Isolated

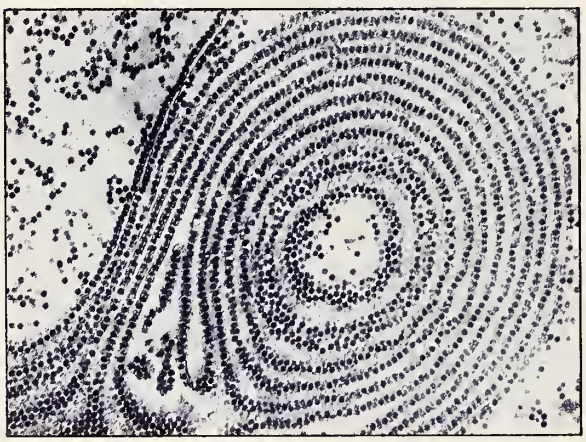
One of the most active areas of virus research is that which seeks to associate viruses with one or more forms of human cancer. During the past year, NIH scientists reported the isolation of a virus from patients with the Wiskott-Aldrich syndrome who have a high cancer rate. The virus, which is a member of a new group of papovaviruses was found in the urine of 3 out of 3 Wiskott-Aldrich patients studied at the NIH Clinical Center. The virus was also found in cultures of the brain tumor which contributed to one patient's death.

A direct relationship of the virus to the tumor has not been established. However, this work, led by Dr. K. K. Takemoto, NIAID, has excited scientists engaged in virus cancer research, since several members of the papovavirus family cause tumors in laboratory animals.

The Wiskott-Aldrich syndrome is an inherited disorder in which patients with the disease have immunologic defects and a high risk of developing cancer. For example, malignancies of the blood-forming system occurred in 6 of 13 boys with the Wiskott-Aldrich syndrome studied at NIH since 1966.

When one of these children developed a brain tumor, virologic and tissue culture studies of the excised tumor were undertaken by NIAID and National Cancer Institute scientists. After 6 weeks, cultures containing tumor fragments underwent changes reminiscent of those seen in cultures infected with a papovavirus known as BK virus (BKV). This virus, found frequently in the urine of immunosuppressed kidney transplant patients, cross reacts with SV40, the well-studied monkey papovavirus which causes tumors in newborn hamsters.





DNA viruses in brain lymphoma cells from patient with Wiskott-Aldrich syndrome.

Further laboratory tests strengthened the suspicion of the scientists that they had isolated in the tumor fragment culture an agent similar to BKV. When they purified the new virus and examined it by electron microscopy, typical papovavirus particles were seen.

A dramatic rise in amounts of antibody to the new virus and to BKV was found to have occurred in the serum of the brain tumor patient as long as a year before clinical signs of his malignancy were noted. Antibody levels continued high through the remainder of the child's life, suggesting a persistent infection and leading the scientists to search for virus in the urine. When samples of urine were collected, spun into pellets, and examined by electron microscopy, large numbers of typical papovavirus particles could be seen.

During the course of these studies, the NIH scientists detected papovaviruses in the urine of two other Clinical Center patients with the Wiskott-Aldrich syndrome. Like the first case, these patients also developed rises in serum antibody titer to BKV and the new papovavirus. One of the patients has now developed cancer and is being carefully studied.

## Treponema Pallidum Consumes Oxygen

Of all bacterial infections, those caused by *Treponema pallidum* and *Neisseriae gonorrhoeae* comprise the most serious public health problem in the United States. Although antibiotics are effective against both these organisms, the venereal diseases they cause occur so frequently that some other method of control is desirable. NIAID launched last year a multidisciplinary approach to the problem and is supporting research designed to develop improved methods of therapy and prevention.

A principal hindrance to research on *T. pallidum*—the spirochete causing syphilis—has been the inability of scientists to grow this bacterium in tissue culture. The organism has always been considered anaerobic, that is, living and growing only in the absence of oxygen. Last year, however, a NIAID grantee—Dr. C. D. Cox at the University of Massachusetts—discovered that *T. pallidum* consumes oxygen at a rate similar to that of another spirochete, *Leptospira*, a known aerobe. Although Dr. Cox's data contradict current theory, the evidence is strong that the organism does indeed require oxygen, and this may be the key to successful cultivation of the bacterium in quantities which will greatly facilitiate research.

In his carefully planned experiments, Dr. Cox measured the concentration of oxygen in cell suspensions containing either *T. pallidum*,

NIAID grantee, C. D. Cox, University of Massachusetts, and his technician, Miriam K. Barber, using the oxygen probe with which they discovered that T. pallidum, the organism causing syphilis, does consume oxygen.

Photo: University of Massachusetts

*Leptospira B* (a known aerobe), or *Spirochaeta stenostrepa* (a known anaerobe). Over a 90 minute period, it could be shown that *T. pallidum* was consuming oxygen at a rate similar to *Leptospira;* and, furthermore, that the means by which oxygen was taken up was the same for *T. pallidum* as for other aerobes. Dr. Cox believes that past findings of the toxicity of air for the organism could be attributed to the accumulation in a culture of oxidized intermediates, such as peroxides, rather than oxygen itself. Anaerobic conditions in a culture would prevent the accumulation of toxic oxidized intermediates, but the absence of oxygen would also prevent growth or reproduction of *T. pallidum.*

### Resistance to Malaria Elucidated

It has long been recognized that, in addition to cellular and humoral immunity, other factors are involved in protecting individuals against infectious diseases. Some people seem to have more "resistance" to a disease, and this resistance is likely to be an inheritable factor.

NIH scientists recently identified, for the first time, a genetic factor which prevents the invasion of human red blood cells by a malaria parasite. The unusual worldwide distribution of this factor—the Duffy negative red blood cell genotype—suggests that it may indeed protect certain human populations against one species of malaria.

Three Duffy genes determine the presence or absence of Duffy a or b antigens on the surface of human red blood cells. The Duffy negative genotype—indicated by the failure of blood cells to react with antibodies to Duffy a or b antigens—is found in 90% of West Africans and 65% of American blacks—groups known to be resistant to infection by the human malaria parasite, *Plasmodium vivax*. The Duffy negative genotype is extremely rare in racial groups susceptible to *P. vivax*. This association between genotype and susceptibility suggests that Duffy antigens a and b may be the actual receptors on red blood cells which allow invasion by *P. vivax.*

Malaria is a daily threat to the lives and well-being of millions in Asia, Africa, and Latin America. This threat might be reduced if scientists could manipulate factors on the malaria parasite, which enable it, in its merozoite form, to invade red blood cells. The invasion stage is essential for continuation of the disease cycle.

The NIH investigators—Drs. Louis H. Miller, Steven J. Mason, and James A. Dvorak, NIAID, and Mary H. McGinniss and Dr. Ivan K. Rothman, NIH Clinical Center Blood Bank—tested red blood cells from normal individuals for their susceptibility to invasion by the monkey malaria parasite, *P. knowlesi.* This parasite had to be used since scientists have not yet been able to grow the human parasite, *P. vivax,* under laboratory conditions.

Only Duffy negative cells resisted invasion by the malaria parasite. Observation of this phenomenon was made possible through use of a unique electro-optical system developed at NIH. The researchers could actually see that, though the parasite attached itself to Duffy negative cells, it was unable to penetrate them. Duffy positive cells (a and/or b), in contrast, were susceptible to invasion by the parasite.

The scientists have other evidence that the Duffy blood antigens are related to susceptibility to malaria. Earlier studies by Dr. Miller and others had shown that treatment with certain enzymes decreased the susceptibility of human red blood cells to invasion by *P. knowlesi.* In their most recent investigations, the scientists showed that this effect is due to the ability of the enzymes to remove Duffy a and b antigens from the cells. Furthermore, blocking the Duffy a antigen with antibody directed specifically against it, greatly reduced its invasion rate.

Since it is now known that the malaria parasite requires specific receptors to invade human red blood cells, it can be assumed that the parasite must contain a corresponding group on its surface that attaches to these receptors. Isolation of this group on the parasite's surface may facilitate malaria vaccine development.

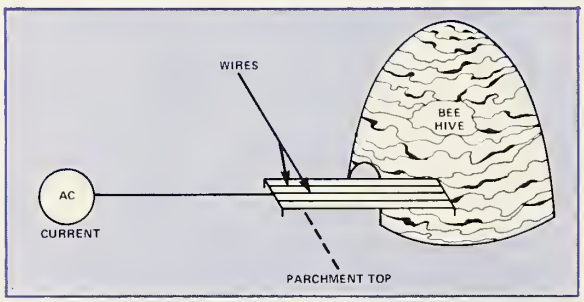
### New Tests for Insect Sting Allergy

Mechanisms of immunity are of as much interest in allergic disease research as in studies of infections. Just as susceptibility to malaria is more pronounced in some persons than in others, the tendency to react positively to some allergens is also an individual matter. For example, each year a significant number of people in the United States die as a result of being stung by such insects as bees, yellow jackets, wasps, and hornets. It has been estimated that as many as 4 in 1,000 persons, stung by one of these insects, have serious systemic (generalized) reactions and, subsequently, live in fear of another sting. Although the clinical symptoms of these reactions sometimes resemble anaphylaxis—a severe allergic shock-type reaction—it is not always possible to differentiate the specifically allergic person from the

non-allergic by skin testing with available extracts of insect bodies.

Now, by using pure insect venoms resulting from improved methods of collection, scientists at two of NIAID's Asthma and Allergic Disease Centers have developed a laboratory test which clearly distinguishes between truly sensitive and normal people. This research has also shown that most systemic reactions to insect stings are allergic in nature and not toxic—thus settling a long-disputed question.






Method developed by NIAID-supported scientists to collect pure bee venom for use in testing for and treating insect allergy. When the bee comes out of the hive onto the metal grid, a mild electrical shock induces the release of venom to the parchment.

In developing their test, Drs. A. K. Sobotka, M. D. Valentine, A. W. Benton, and L. M. Lichtenstein at Johns Hopkins University, Baltimore, first obtained white blood cells (leukocytes) from 16 patients who had experienced symptoms suggestive of systemic anaphylaxis following an insect sting. White cells from 12 hospital employees with no history of insect allergy were also studied. When the white cells of the 16 patients were challenged in the laboratory with several pure venoms, all but 3 samples released histamine—a chemical mediator agent involved in the allergic response. No normal individual had histamine released from the tested leukocytes. Two of the 3 patients whose white cells failed to release histamine had been stung by unidentified insects whose venoms might not have been among those available for testing.

The patterns of the patients' reactions to the various venoms showed only limited cross-reactivity between the substances. Seven patients reacted only to the yellow jacket/hornet family of venoms; 3 only to honeybee venom; and 3 to both classes. This observation puts into question the common practice of using a mixture of venoms prepared from the several insect families to test and treat patients.

Even more questionable is the use of whole body insect extracts for skin testing and immunotherapy. In most instances, patients whose leukocytes readily released histamine in reactions to the purified venoms failed to respond to the commercially-available whole body extracts.

Directly related to this problem is a report by Dr. Sobotka and her co-workers and by Drs. C. C. Charvejasrn, J. I. Wypych, R. E. Reisman, and C. E. Arbesman, State University of New York, Buffalo, that they have found that bee venom alone contains a specific antigen not present in significant amounts in whole body extracts. This substance, called phospholipase A, is a major antigenic component of the venom. Since the Johns Hopkins investigators have noted that sera from 2 patients immunized with purified bee venom contained antibodies which could block, in the test tube, the allergic response of sensitized leukocytes, it has been suggested that immunization with honeybee venom be monitored in a group of appropriate persons.

## Penicillin Allergy Blood Test Developed

Improvement of testing techniques is also the subject of research on drug allergies. An NIAID grantee at Johns Hopkins University recently reported progress in developing a reliable blood test for allergy to penicillin, using the RAST (radioallergosorbent test) technique. His preliminary results with a limited number of blood samples suggest that this assay for antibodies of the IgE class correlates very well with skin testing and is of sufficient sensitivity to detect patients who are likely to have immediate or accelerated penicillin reactions if given the drug for treatment of their infection.

Recent clinical studies have established the usefulness of skin testing with two penicillin antigen preparations: penicilloyl-polylysine (PPL); and a group of highly reactive penicillin metabolites known as the minor determinant mixture (MDM). A positive skin test with either of these two reagents is indicative of a significant current risk of serious reactions to penicillin, and is much more reliable than a history of prior hypersensitivity.

In spite of the reliability of these skin tests, there are a number of compelling reasons for attempting to develop a means for determining penicillin sensitivity by examination of blood sera alone. First, there is a small but definite risk of skin testing with drugs and their metabolites in highly sensitive patients. Deaths from penicillin skin testing have been reported. Secondly, the penicillin skin testing reagents are not yet generally available and their use requires some

degree of skill and experience. And, third, a serological assay, such as RAST, would enable investigators to screen large numbers of patients for penicillin IgE antibodies and could result in significant reduction in illness and death among patients not suspected of being sensitive to the drug. Finally, some serological assays can often be more precise and reproducible than single dose skin testing.

Swedish investigators (Wide and Juhlin) were the first to show that the RAST technique can be employed to detect circulating IgE antibodies directed against the major penicillin antigen determinant—the penicilloyl group. Dr. Wide's lead was followed up by Dr. N. Franklin Adkinson, Jr., Johns Hopkins University, during the past 2 years.

Dr. Adkinson and his co-workers first perfected a technique (the RAST elution test) whereby absolute quantities of specific IgE antibodies in a serum sample can be determined; then, working with a limited number of patients, the investigators compared results of the RAST test for penicillin antibodies with results of skin tests and histories of penicillin allergy.

Eight patients all had positive histories, skin tests, and RASTs. Three patients had positive PPL skin tests, histories, and RASTs but negative MDM skin tests, confirming the specificity of the assay for the minor antigen determinant. This specificity was further confirmed in a group of patients whose MDM skin tests were positive but whose PPL skin tests and RASTs were negative. Twelve individuals with positive histories of prior penicillin reaction but negative skin tests were also assayed and all were negative.

As controls, 18 persons with no history of prior reaction all had negative RASTs.

Although Dr. Adkinson and his group consider these results encouraging, they point out that they have yet to tackle the problem of developing a serological test for penicillin antibodies to the minor antigenic determinants. Successful solution to this problem will require sophisticated immunochemistry as well as access to a large number of sera from patients who are MDM skin test positive. This work is currently in progress.

### Identifying Origins of Malignant Cells

Immunology research also has application to cancer. For example, the origin of malignant white blood cells can now be identified by NIH immunologists using markers on the cells' surfaces. Results of research by NIAID and National Cancer Institute investigators should lead to a new classification of human lymphoid tumors, thereby improving the diagnosis of patients with such disorders.

Malignant lymphoid cells preserve many characteristics of "normal" immune response cells—the T-cells (thymus-derived lymphocytes), B-cells (bone marrow-derived lymphocytes), and monocytes. The presence or absence of these characteristics, or receptors, can be used to identify cell populations.

Using several techniques (including their own) which identify receptors on normal cells, Drs. Ira Green, Ethan M. Shevach, and Michael Frank, NIAID, and Drs. Elaine S. Jaffe, Richard L. Edelson, and Costan W. Berard, NCI, studied malignant cells either in suspension or in extremely thin slices of frozen tissues. Malignant cells from patients with such little understood diseases as "hairy cell leukemia" and histiocytic medullary reticulosis were found to be monocyte proliferations; while poorly differentiated lymphoma and mycosis fungoides were determined to be T-cell disorders.

Abnormal cells of Sezary syndrome patients also exhibited T-cell characteristics, including the production of MIF—a chemical generated by sensitized lymphocytes when they interact with antigen. This finding confirms the belief of many scientists that Sezary syndrome and mycosis fungoides—both lymphomas with malignant involvement of the skin—are closely related. Identification of its T-cell origin should enable accurate diagnosis of Sezary syndrome, which has often been confused with chronic lymphocytic leukemia, a B-cell disorder.

Although these techniques are still in their infancy, the Bethesda scientists hope that determination of the precise origin of lymphoma cells will provide insights into the factors that cause the tumors. In addition, the ability of these tumors to produce large uniform population of monocytes, or T-, or B-cells will provide researchers with valuable tools for further studies of both normal and malignant immune response cells. Abnormal T-cells could also be used to isolate human T-cell lymphokines—chemical mediators of cellular immunity.

# National Institute of Arthritis, Metabolism, and Digestive Diseases

Drs. Paul H. Plotz (l.) and Robert Kimberly of NIAMDD examine x-rays of patient's hands with rheumatoid arthritis.



## Search for Control and Understanding of Arthritis

Understanding of a disease process usually offers valuable guidance in the search for effective remedies. The limited understanding of rheumatoid arthritis has meant that the search for remedial agents must proceed largely through the process of trial and error. Once successful agents are found, they become the focus of intense investigation, and understanding the mechanism by which a newly discovered treatment produces its effect hopefully leads to fresh perspectives on this disease.

Yale University investigators Drs. Stephen Malawista and Daniel Wright, working with support from the National Institute of Arthritis, Metabolism, and Digestive Diseases, report that the anti-inflammatory drug colchicine, long recognized as very effective in relief of the acute arthritis of gout, acts by preventing release of destructive enzymes from "policing" and scavenging white blood cells. Release of these enzymes, normally stored as stable intracellular particles called "lysosomes," may well be the final common path in production of cartilage destruction and connective tissue overgrowth in arthritis-damaged joints.

The researchers have studied three anti-inflammatory agents, and have shown that colchicine and cortisol produce inhibition in human leukocytes, which may account in part for their anti-inflammatory effects. These two agents also significantly inhibited release, from the white blood cells, of acid phosphatase and cathepsin (which have tissue-destroying or "digesting" capabilities). Sodium salicylate, on the other hand, failed to suppress intracellular release of lysozyme, or intracellular loss of acid phosphatase or cathepsin. This inquiry into the cellular responses of these agents establishes a valuable link between old, well-tested knowledge of treatment and understanding of disease mechanisms provided by new knowledge of cellular metabolism.

Another empirical approach to treatment now being examined from the perspective of new knowledge is that of aiming for prompt control of inflammation. A fresh vantage point is being provided by the observations of Dr. Edward D. Harris, Jr., NIAMDD grantee at Dartmouth College. His research has shown that human cartilage collagen is relatively resistant to destruction by collagenase, but that the rate of destruction is affected by changes in temperatures. Collagenase is a naturally occurring enzyme which catalyzes the destruction of collagen, the main supportive protein of cartilage and connective tissue. It has long been believed that collagenase plays a primary role in the joint destruction characteristic of rheumatoid arthritis, but solid evidence of this effect has been lacking.

Dr. Harris has found that collagen breakdown is four times greater than normal at the elevated temperatures found within knee joints afflicted with rheumatoid arthritis, suggesting that part of the benefit from anti-inflammatory therapy may result from lowering the temperature of the affected joint.

It is known that treatment of inflamed joints with induced heat, such as diathermy, increases internal joint temperature and almost invariably aggravates the inflammation. The present finding provides a rationale for effective local suppression of joint inflammation, particularly by intraarticular injection of potent steroid drugs, that is more scientifically acceptable than mere symptomatic relief of pain and swelling.

Another grantee of NIAMDD, Dr. Paul I. Terasaki of UCLA, and his associates, have demonstrated an increased prevalence of the histocompatibility antigen W27 in patients with juvenile rheumatoid arthritis. This disorder of children presents features that mark it as a separate disease from classic rheumatoid arthritis, particularly when high fever and skin eruption are present without the elevation of rheumatoid factor characteristic of adult rheumatoid disease.

The histocompatibility antigen W27 (one of the many immunologic "markers" that distinguish different tissue "types") is present in 6 percent of the normal population, in 80 to 90 percent of patients with ankylosing spondylitis, in 76 to 96 percent of patients with Reiter's disease, and in 55 percent of patients with acute anterior uveitis. Reiter's disease is characterized by a simultaneous inflammation of the joints and the urethra, and uveitis in the eye. Because of the clinical similarities of ankylosing spondylitis and juvenile rheumatoid arthritis, and the high prevalence of anterior uveitis in the latter, W27 was looked for in patients with juvenile rheumatoid arthritis.

Forty-two percent of the patients with juvenile rheumatoid arthritis were found to have the W27 antigen. Although Reiter's disease, ankylosing spondylitis, and juvenile rheumatoid arthritis are three distinct diseases, the high prevalence of W27 in these closely related pathologic entities suggests the same or similar etiologic agents,

possibly of an infectious nature. The evidence suggests that there is a common origin to various arthritic diseases, such as inherited susceptibility to infectious agents which are yet to be identified.

### The Promise of Somatostatin

For many years after the discovery of insulin in 1922, it was thought that diabetes resulted simply from the inability of the pancreas to produce and release sufficient amounts of this hormone. Researchers now recognize that they are dealing with a complex, multifaceted disease, and not with a relatively simple metabolic disturbance.



In 1971, for example, an Institute-supported scientist at the University of Texas, Dallas, reported that diabetes is characterized by excessive glucagon secretion, in addition to insulin deficiency, and thus may be a bihormonal disorder. Glucagon, a hormone secreted by pancreatic alpha cells, is antagonistic to the actions of insulin in that it stimulates release of glucose from the liver and tends to elevate blood glucose levels.

This finding initiated a search for an agent which might suppress such elevated glucagon levels, and which, therefore, might be of therapeutic value in diabetes. At the University of California, San Francisco, Dr. Peter Forsham and associates, working with NIAMDD support, have discovered that a hormone produced in the hypothalamus portion of the brain—somatostatin—can lower plasma glucagon and glucose levels in healthy individuals.

Turning to diabetic patients, the investigators found that infusion of somatostatin decreases plasma glucagon levels by 50 percent and the abnormally elevated plasma glucose levels by 25 percent. Moreover, somatostatin combined with insulin was found to be more effective than insulin alone in diminishing the sharply elevated blood glucose levels which occur in diabetic patients immediately after eating a meal.

Unfortunately, somatostatin lowers increased plasma glucagon and glucose levels in diabetic patients only temporarily. It is believed, however, that this hypothalamic hormone, in some longer-acting form, may prove to be a useful adjunct to insulin in treatment of diabetes.

### Obesity, Cell Receptors and Insulin

In adults, obesity and diabetes are often related and have several metabolic features in common. Obesity, like diabetes, is frequently accompanied by elevated blood levels of glucose, excessive amounts of insulin, and generalized resistance to the effects of this hormone. It is, however, but one of many disease states, including many diabetic syndromes, in which cell responsiveness to insulin is impaired. This condition contrasts with the more dramatic juvenile diabetes, where a deficiency of insulin production is the primary defect.

Insulin is manufactured in the pancreas, released into the blood stream, and distributed to cells throughout the body. For insulin to activate a cell, the hormone first binds to specific receptors located on the cell surface. There receptors serve two major functions: 1) they act to distinguish insulin from other molecules to which they are exposed, and then bind the insulin tightly at the cell surface; 2) the combination of hormone with receptor initiates a signal which activates intracellular processes characteristic of insulin action.

NIAMDD's Dr. Jesse Roth, and associates, recently devised methods to measure precisely the binding of insulin to specific receptors on cells. They found that rapid changes occur in both the number of receptors per unit of cell surface, and in the tightness with which each receptor binds insulin. These alterations, which were largely unsuspected, influence the effectiveness of a given amount of insulin. In obese persons, and experimentally in obese mice, the number of insulin receptors per cell was found to be subnormal, which accounts for much of the decreased responsiveness to this hormone.

Moreover, when overeating stops, blood glucose and insulin levels return to normal, the number of insulin receptors on the cell increases, and sensitivity to the effects of insulin becomes normal. Thus, there is a new sound scientific basis for diet therapy of obese diabetics, and it is both effective and safe.

Application of these new procedures has led to the discovery that receptors play a role in at least several other disease states. Part of the resistance to insulin treatment in uncontrolled diabetic acidosis is due to a decrease in the tightness with which receptors bind insulin. Elevated levels of adrenal steroid hormones, whether natural or induced, often cause derangements of blood glucose and impaired responsiveness to insulin that is due to alterations in the cell receptors for insulin.

The investigators recently studied a group of patients with diabetes who were extremely resistant to massive doses of insulin; all had extreme impairment of insulin binding to

receptors. Conversely, in several disorders of the pituitary and adrenal glands characterized by supernormal sensitivity to insulin, receptors for insulin are increased in number.

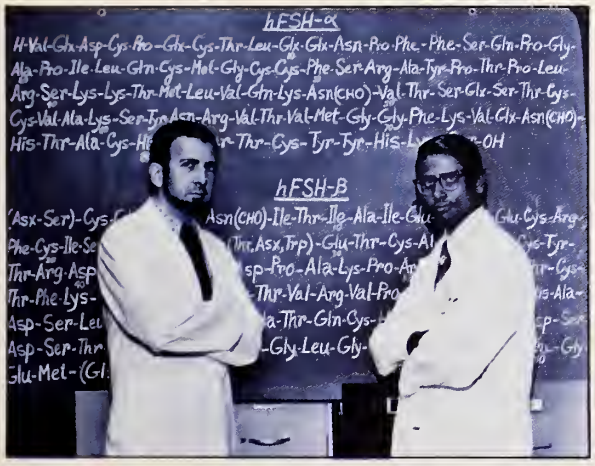
The therapeutic implications of this research may extend beyond insulin to all types of hormonal diseases, even including major diseases of other organ systems such as coronary artery disease and several forms of cancer, in which hormones play a significant role.

### Unraveling the Structure of FSH

The human pituitary or "master" gland in the brain produces a great variety of hormones that are vital to many body functions. Among these are two so-called sex hormones—luteinizing hormone (LH) and follicle-stimulating hormone (FSH).

In recent years investigators have determined the chemical structure of LH. Now, NIAMDD-supported scientists at the Harbor General Hospital, Torrance, California, have reported that they have determined the amino acid sequence of FSH.

Previous attempts to unravel the structure of FSH were hampered by a lack of human pituitary glands, which are obtainable only at autopsy. The organs used by the present investigators, Drs. Albert F. Parlow and Basudev Shome, were provided by the National Pituitary Agency, a pituitary gland collecting and processing entity organized and funded by a contract from the NIAMDD. With a ready supply of human FSH thus available, the investigators, through a series of sophisticated chemical procedures, were able to determine the hormone's chemical structure.




Drs. Albert F. Parlow (l.) and Basudev Shome display their diagram of the amino acid sequence of follicle stimulating hormone (FSH).

Ultimate development of a male contraceptive could result from immunization of men with anti-FSH globulins, thus preventing sperm production that is regulated by FSH. Infertility resulting from inadequate production of FSH in both men and women may also be corrected ultimately through administration of FSH, although large-scale synthesis of the large, complex protein is unlikely in the near future.

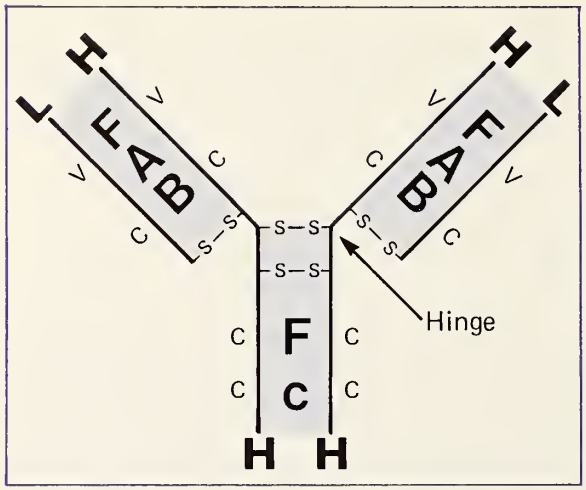
### Antibody Structure

One of the major questions concerning the immune system has been the molecular nature of antibodies and the mechanism by which they bind to antigen. Recent X-ray diffraction studies in several laboratories, including the Laboratory of Molecular Biology, NIAMDD, have gone a long way toward providing a general picture of the three-dimensional structure of antibodies.

These studies, which have so far been limited to fragments of antibody molecules, have established that there is a basic folding pattern that is common to all antibodies and consists of globular units (or domains) of approximately 110 amino acids. The whole molecule, which contains four polypeptide chains (2 light and 2 heavy chains) is built up of 12 of these domains associated in pairs.

The antigen binding sites of the antibody are formed by pairing the domains at one end of the light and heavy chains. The surface formed at the junction of these domains is made up of amino acids that vary greatly from one antibody to another whereas the remainder of the amino acids are relatively constant. The high variability of these amino acid sites, previously observed by Drs. Wu and Kabat of Columbia University, provides the molecular basis for the wide range of antibody specificity.

The NIAMDD scientists, Drs. David Davies, Eduardo Padlan, and Anne Segal, working in collaboration with Drs. Michael Potter and Stuart Rudikoff of the National Cancer Institute, were able to grow crystals of homogeneous antibody fragments from the mouse. X-ray diffraction analysis of these crystals resulted in a picture of the molecular arrangement in three dimensions. This particular antibody specifically binds the small hapten, phosphorylcholine. Crystals were soaked in phosphorylcholine, which diffused in and bound to the antibody fragment. Extensive data collection and computer analysis then revealed the location of the phosphorylcholine binding site and permitted the identification of the specific amino acids




The 3-lobed structure of antibody was first described by Dr. Rodney R. Porter of Oxford University in 1959. The structure consists of 2 Fabs (Fragments, antigen-binding) and an Fc (Fragment, crystalline) segment.

involved in binding. It has been observed that these amino acids are present at the same location in other mouse antibodies that bind phosphorylcholine.

An understanding of the mechanism of antibody-antigen interaction is central to our understanding of the immune system and the resistance to disease of higher organisms.

### Metabolic Studies in Skylab Orbital Flights

The Space Age has opened vast new fields of research in a variety of disciplines, some of which did not even exist prior to the 1960's. It has stimulated investigations in the biomedical as well as the physical sciences. What are the effects of prolonged space flight on the human body? How can metabolic imbalances produced by periods of weightlessness be corrected and prevented? These and other questions pose new problems to the spaceage scientist.

NIAMDD Director and investigator Dr. G. Donald Whedon has described significant in-flight losses of calcium, nitrogen and phosphorus from bones and muscle of Skylab astronauts as a result of living without gravitational stress. This suggests that prolonged space flights of many months duration could endanger the human musculoskeletal system unless effective protective measures can be developed.

Studies of all participants in the three Skylab flights of 28, 60 and 84 days, respectively, called for rigorous feeding and waste collection regimens. These required fairly constant dietary intake, continuous 24-hour urine collections and total fecal collections for 21 to 31 days before each flight, throughout each flight, and for 17 to 18 days after each flight, for a total of 909 man-days of metabolic study.

Although total urinary calcium losses were moderate in relation to the whole skeleton, over a period of many months such losses could endanger the strength of critical areas of the body's long bones. Similarly, the increased losses of urinary nitrogen and phosphorus reflected substantial loss of muscle tissue which could be clearly observed in the astronauts' legs. Mineral and nitrogen balances returned to normal after the astronauts returned to earth.

Dr. Whedon, and associates at the Veterans Administration Hospital, Sepulveda, California, and the NASA-Johnson Space Center, Houston, Texas, concluded that although prolonged space flight of 6 to 9 months might reasonably be predicted as "safe," an 18- to 36-month trip (the time required to go to Mars and return) could have serious effects on bone and muscle unless protective measures can be developed.

NIAMDD's Jeanne Reid, research dietitian, enjoys a break from the tight Gemini VII schedule with astronauts James Lovell (l.) and Frank Borman at Cape Canaveral.

### Dissolving Gallstones

Little is known about how gallstones are formed, but gallstone disease ranks fifth among the causes of hospitalization, striking about 15 million Americans. Surgery is the standard form of treatment and some 375,000 gallbladder operations are performed each year at an average cost of $2000 each. Inasmuch as many gallstone patients are poor surgical risks and an estimated 6,000 patients die each year as a result of such surgery, an alternate way of controlling the disease has long been sought.

In recent years, NIAMDD grantees Drs. Johnson L. Thistle and Alan F. Hofman and associates at

the Mayo Clinic, in Rochester, Minnesota, discovered that long-term oral administration of a primary bile acid, chenodeoxycholic acid, can result in dissolution of long-standing cholesterol gallstones. Thus, it would appear that prolonged administration of this bile acid may offer an alternative medical treatment for many patients with gallstones who previously had no choice but surgery. Chemical dissolution of gallstones may also possibly help prevent gallstones from beginning or continuing to develop in patients diagnosed as being at special risk.

More recently, the same investigators tried to determine if patients treated successfully with this agent might relapse when therapy is discontinued. As it turned out, when chenodeoxycholic acid administration was stopped, bile from such patients reverted to its pretreatment state of being supersaturated with cholesterol, altered bile acid composition induced by therapy was reversed, and new gallstones developed in several patients. This indicates that any therapeutic intervention in patients known to have cholesterol gallstones and any preventive administration of chenodeoxycholic acid in patients at special risk to develop them, may have to be followed by a continuous maintenance treatment with this bile salt.

These observations in a relatively small number of patients showed the need for controlled long-term chenodeoxycholic acid therapy, as well as the possible need for a prophylactic maintenance regimen after existing cholesterol gallstones have been dissolved successfully. In order to determine further the feasibility of chenodeoxycholic acid therapy, the NIAMDD has awarded a contract to Cedars-Sinai Medical Center in Los Angeles to supervise and participate in a 5-year, multi-million dollar testing project. About 1000 patients are now being treated at nine medical centers.

### Enzymes Pitted Against Toxic Compounds

Our environment is fraught with toxic compounds to which we are continually subjected—substances ingested in our food, inhaled with smog, and taken in the form of drugs to treat illness. The human body also produces its own toxins as normal breakdown products of tissue components; for example, the bile pigment, bilirubin, is a toxic product of heme a constituent of hemoglobin of the blood. How, in the face of this constant barrage of toxic substances, do we escape being poisoned and manage to remain viable?

The answer lies in a number of enzymes which, by oxidizing, rearranging and combining these poisons with other compounds ("conjugation"), prepare them for rapid elimination in bile or urine. One group of detoxification enzymes, the $P_{450}$-oxidase system, can itself be dangerous in that it produces a group of reactive oxidation products, the arene oxides, some of which are known to be potent carcinogens. Research by NIAMDD's Dr. Donald Jerina and associates is concerned with enzymes that hydrolyze these arene oxides, *i.e.*, proteins which catalyze the reaction of water and compounds bearing an epoxide linkage.



Another group at NIAMDD, Dr. William Jakoby and his colleagues have now isolated a different group of enzymes, the glutathione S-transferases, in homogeneous form from both human and rat liver. These enzymes catalyze the reaction of glutathione, a normal body constituent, with arene oxides as well as with a vast range of other compounds including drugs, pesticides and industrial contaminants. These reactions result in the formation of thioethers which are generally non-toxic, and they and their breakdown products are readily excreted from the body uneventfully.

The glutathione transferases serve a second function in that they bind to a great many compounds that are not converted to a glutathione conjugate. Since these enzymes comprise about 2 percent of the soluble protein of human liver—and 10 percent of rat liver—the capability of binding a large amount of such toxic substances as bilirubin is itself a detoxification function and forms part of the complex pattern of storage and transport necessary for elimination.

These enzymes perform yet a third role in detoxification. If the compound bound to them is a very reactive one (and this is the case for certain carcinogens) it will react directly with the enzyme and form a permanent link with it. This is a form of suicide for the enzyme since the resultant linkage inactivates further catalytic and binding activity.

The study of these enzymes not only demonstrates the operation of a major group of detoxification processes in man, but it also provides the means for investigating properties basic to all enzymes: the interaction of binding, catalysis, and reaction with protein. These properties are vital to the maintenance of harmonious body functions, particularly in light of man's existence in a toxic world.

## New Device for Edema

Current treatment of edema (waterlogging of tissues), regardless of its cause, employs diets restricted in sodium and occasionally in fluids, as well as therapy with drugs which stimulate the excretion of urine. Such treatment usually requires days to weeks to correct the body's fluid overload, while a few unresponsive patients may require salt and water removal with peritoneal dialysis or an artificial kidney.



Dr. Lee W. Henderson and his associates of the University of Pennsylvania, working under a contract from the NIAMDD's Artificial Kidney—Chronic Uremia Program, have reported clinical trials of a new device that efficiently and rapidly ultrafilters the blood of patients on chronic hemodialysis to remove excess water and sodium.

The new ultrafiltration cell inexpensively removes excess sodium and water from fluid-overloaded patients on chronic hemodialysis; this device can be plugged into the extracorporeal circuit of an artificial kidney in dialysis patients, or it can be used as a separate unit in other patients with refractory edematous states. Conceivably, one or two ultrafiltration treatments of 6 to 8 hours could replace days to weeks of costly hospitalization to "dry out" an edematous patient.

Therapy with the new device is simpler and safer than fluid removal by peritoneal dialysis or hemodialysis. No serious complications, such as clotting, occurred in over 100 clinical trials. Although used to date only in a dialysis setting, it is suggested that such therapy could logically be extended to patients with refractory chronic edematous states (such as chronic congestive heart failure) or with pulmonary edema, and could conceivably replace costly and prolonged hospitalization with one or two relatively brief ultrafiltration treatments.

## "Photochemotherapy" for Psoriasis

During Biblical times, victims of psoriasis were regarded as lepers and were forced to carry a bell to warn people of their presence. Even today, the reddish patches of skin covered with whitish scales that plague almost 8 million Americans, cause immeasurable embarrassment and discomfort, although the belief that psoriasis is contagious has long since been dispelled. Nevertheless, the search for a lasting and effective treatment for psoriasis is almost as old as the disease itself.

The management of *generalized* psoriasis, whether by topical or systemic therapy, has not been satisfactory for those victims who suffer from this frustrating and unsightly skin disease. Use of methotrexate, the most effective systemic agent to date, may be associated with severe liver toxicity, while ultraviolet radiation following local application of crude coal tar requires hospitalization for up to 2 weeks.

Now, Dr. Thomas B. Fitzpatrick and associates at the Massachusetts General Hospital in Boston have achieved complete clearing of psoriasis in a significant number of patients with an experimental technique that combines a newly developed light device, which emits special long-wave ultraviolet light, and oral administration of methoxsalen, a drug that Egyptians have used since ancient times. The interaction of light and drug termed "photochemotherapy" by the investigators, presumably inhibits epidermal DNA systhesis and, thus retards the well-known rapid proliferation of the outer skin layer in psoriasis.

This potentially promising technique could be commercially available within a few years if a 9-month, 10-university research center trial of 1,000 patients shows similar promising results with no side effects. The new therapy is not a permanent *cure* for psoriasis, which is a genetic disease inherited in a poorly understood pattern. Yet, treatments repeated at intervals have kept patients free of psoriasis skin patches for up to 10 months, thus offering new hope for those afflicted with this chronic disease.

## Viruses and Tumors

Simian virus 40 (SV40) is a DNA virus which produces tumors in hamsters. A closely related virus produces a fatal presenile dementia in man known as progressive multifocal leukoencephalopathy (PML). Since the DNA content of SV40 is so small, an analysis of this virus should readily yield information as to how it can produce tumors.

The growth behavior of SV40 is very different in monkey kidney cells than in other cell types. In monkey cells the virus replicates its DNA and then synthesizes the proteins that are used to encapsulate (coat) the DNA, finally killing the cell and releasing about 1,000 progeny virus particles. In other cells the virus becomes incorporated into the host DNA and causes changes in the growth behavior of the host cell. These characteristic changes in growth properties in tissue culture—rapid growth in medium with low serum, ability to grow in agar suspensions, and ability to overgrow a monolayer of

normal cells—are referred to as transformation. Very few virus particles are released from transformed cells. A close, but not absolute correlation exists between transformation and tumorgeneity, *i.e.*, other factors including immunologic competence affect the tumorigenicity of transformed cells.

A genetic analysis by NIAMDD's Drs. Robert Martin and Janice Chou has shown that SV40 contains only three genes. Two have tentatively been identified as corresponding to the proteins involved in the formation of the viral capsid (coat) proteins. These genes are generally not expressed upon transformation. The third gene appears to be responsible for transformation and must be expressed continually if a host cell is to remain transformed. It is also known that this same gene is responsible for the initiation of viral DNA synthesis in monkey cells. It has therefore been suggested that these two facts are related and that transformation by SV40 is the result of aberrant DNA synthesis in the host. Attempts are currently underway to define the biochemical reaction carried out by the product of this gene, in an effort to fully understand how SV40 causes transformation.

### Hemoglobin S and Sickle Cell Disease

Major efforts are under way to develop therapeutic measures for sickle cell disease, a severe inherited anemia. Sickle cell disease occurs when an abnormal type of hemoglobin is present in red blood cells (hemoglobin is the molecule which enables red blood cells to carry oxygen throughout the body).

The NIAMDD's Drs. James Hofrichter, William Eaton and Philip Ross have been investigating the physical properties of this abnormal hemoglobin, called hemoglobin S. Release of oxygen by the red cells results in aggregation of the hemoglobin S molecules—a process called gelation—to form bundles of fibers that cause the cells to become rigid and distorted. These "sickled" cells are more readily destroyed, causing a chronic anemia. Sickled cells may also become lodged in the narrow blood vessels, block blood circulation, and thereby cause tissue damage and pain in different parts of the body. Such acute episodes occur intermittently and are known as sickle cell "crises."

If gelation of hemoglobin S can be prevented, then the clinical manifestations of the disease can be prevented. Finding an effective therapy for sickle cell disease thus becomes a problem of inhibiting a single, well-defined molecular process.

The Institute scientists have developed several techniques to study gelation of hemoglobin S in the test tube. They discovered that the rate of gelation shows very unusual behavior. Before gelation begins, there is a delay period, followed by a rapid approach to the final, "sickled" state. Surprisingly, the length of the delay period depends very critically on the hemoglobin S concentration; a decrease of only two percent in the hemoglobin concentration, for example, lengthens the delay time by 80 percent. They also found that the delay time is very sensitive to temperature and other physiological variables.



These findings have been explained by a model in which energy input is required initially to form small aggregates of hemoglobin S. After a critical size of about 30 molecules is reached, the reaction proceeds spontaneously at a rapid rate. The model also explains the observation that the delay time is controlled by the ratio of total hemoglobin S concentration to its solubility; lowering the solubility acts similarly to raising the concentration.

There are clear implications of this work for devising a rational therapy for sickle cell disease. Because the time required for gelation can become comparable to the time it takes a red cell to traverse the narrow blood vessels, actual sickling could be markedly reduced by lengthening the delay time. Delay time could be lengthened by decreasing intracellular hemoglobin concentration, or by increasing solubility. The investigators now are screening a variety of agents for their ability to lengthen the delay time, in order to design a molecule that could be of therapeutic value. In addition, clinical studies are being planned to translate their basic findings to direct application.

## Abnormal Sleep Patterns and SIDS

About ten thousand infants each year become victims of crib-death, or Sudden Infant Death Syndrome (SIDS). Typically an apparently healthy infant, usually between 1 and 7 months of age, is put to bed without any indication that something is amiss, although he may have a slight respiratory infection. Later the child is found dead in his crib, but medical examination and autopsy are unable to uncover any lesion, infection, or disorder accounting for his death. The National Institute of Child Health and Human Development is devoting a major research effort to identifying the etiology of this disease.

Dr. Alfred Steinschneider of the State University of New York (SUNY), Upstate Medical Center, Syracuse, and his co-workers are studying the possibility that sleep apnea, a temporary periodic cessation of breathing, may in combination with other physiological events be a precipitating factor in crib death. Prolonged apneic episodes were found in association with marked changes in other physiological functions. Some premature babies experienced sudden prolonged periods of sleep apnea accompanied by cyanosis and severe bradycardia and a cardiac arrhythmia (slow and erratic heartbeat), demanding immediate intervention. Some infants suffered repeated episodes of this nature. These observations suggest that the severity of an episode of sleep apnea may be related to more profound alterations of physiological function.

The investigators first noted the possible association of sleep apnea with sudden infant death syndrome while studying five infants who had been referred to the Center because of recurrent cyanotic and apneic episodes. During observation in the hospital sleep laboratory all of the infants had frequent brief apneic episodes. On the hospital ward they had a number of prolonged apneic and cyanotic episodes (15 seconds or longer), some of which were of sufficient duration and severity to prompt vigorous intervention. In Dr. Steinschneider's words, "the babies merely slept, stopped breathing, and turned blue. They were not struggling." This prolonged cessation of breathing was most often associated with upper respiratory infection. Two of the infants subsequently died at home and medical findings on autopsy were similar to those found in sudden infant death syndrome.

The researchers subsequently focused on respiratory and cardiac rate activity during sleep. The decision to examine sleep was based on the recognition that dramatic, although transient, changes can occur in nervous regulatory mechanisms of respiratory or heart activity—("autonomic storm")—while an infant is sleeping. The sudden onset of sleep apnea could thus occur in an otherwise normal infant, but might not always result in death.

In order to examine this hypothesis, variables associated with sudden infant death syndrome, including sleep, postnatal age, low birthweight, and nasopharyngitis, and the relationship of each to apnea, were systematically studied.



One study examined the number and duration of apneic pauses during a "standard" nap in a sleep laboratory. Infants were carefully observed on the ward for at least a week and placed into one of four categories: infants who had prolonged sleep apnea; infants who had no difficulty during sleep, but had been observed to become apneic or cyanotic during feeding; infants who had both prolonged apnea and feeding difficulties; and infants who had neither feeding nor sleep problems, but who had seizures, breathholding "spells," or skin color changes. When these infants were monitored in the laboratory during a single "standard" nap, two groups of babies had more frequent and longer episodes—the same groups observers had characterized as having prolonged apnea during sleep, and during sleep and feeding. Thus, the laboratory "nap" method had discriminated those infants at risk for prolonged apnea and demonstrated its reliability as a possible diagnostic tool. It was found that REM (rapid eye movement) sleep—the sleep characterized by increased variability of respiratory rate, heart rate, and other physiological changes—is associated with more frequent apnea. In contrast, during non-rapid eye movement (NREM) sleep, heart rate and breathing are slow and regular.

These findings indicate that the same basic neurophysiological mechanism may be responsible for both prolonged sleep apnea and the much briefer apnea frequently noted during routine sleep studies.

Preliminary results showed that among month-old infants of varying birthweights, low birthweight infants were more likly to have longer and more frequent apneic episodes. A previous longitudinal study on a small group of premature babies had established that apnea increased during the first few weeks of life and then decreased as the child matured. In that study, REM sleep again was associated with more

frequent apneic episodes. However, duration was unaffected by sleep stage, thus suggesting that apnea may be best understood as subject to two sets of neurophysiologic mechanisms, one responsible for the initiation of the episode and the other limiting its duration.

A previous observation that upper respiratory illness increased the likelihood of prolonged sleep apnea gained substantial support in these studies when a greater daily evidence of sleep apnea during periods when infants were described as having "colds" was reported.



These and related investigation have provided new insights on sleep apnea in infants and its possible relationship to SIDS. In addition, use of monitoring systems during the course of the studies has made it possible to identify many infants at high risk for apnea.

### Effects of Exercise During Pregnancy

How active can a pregnant woman be without risking harm to her unborn child? Does excessive activity increase the likelihood of fetal distress during labor and delivery? It is well established that pregnancy imposes significant burdens on maternal circulation and respiration, but much of the data regarding oxygen requirement of the fetus and the effects of maternal exercise on it have been incomplete.

Lack of information is especially critical when doctors counsel women whose response to exercise may be altered by pulmonary, cardiovascular, or hematological disease. How much should these women modify their activities to provide a favorable fetal environment?

NICHD-supported researchers, Dr. James Metcalfe and his associates at the University of Oregon Medical School, Portland, are measuring the effects of pregnancy upon maternal oxygen consumption during standard exercise to find answers to some of these questions.

The Oregon scientists studied normal women, those carrying twins, and those with obesity problems, anemia, or heart disease. Rates of maternal oxygen consumption were measured at several periods during their pregnancies while the women were at rest, performing steady exercise, and during recovery from activity. The women volunteers were followed from 3 to 6 months after giving birth, and oxygen consumption was again measured. Mothers' breathing rates were recorded and both maternal and fetal heart rates were monitored.

Exercises were performed on a treadmill and on bicycles. They were designed so that the intensities of both types of exercise were similar, and mild enough so as not to tax those whose activities were limited by disease.

Responses of the women with twin pregnancies, obesity, anemia or heart disease were compared with those of normal women with single pregnancies. Comparisons were made between weightbearing exercise on the treadmill and non-weightbearing bicycle exercise in order to define differences in the two exercises in terms of their metabolic costs. In addition, the scientists studied the relationship between the mother's response and fetal heart rate during maternal exercise and compared the relationship to the weight of the baby at birth.

Dr. Metcalfe's findings suggest that normal maternal tissues may sustain some degree of oxygen deprivation during exercise. Furthermore, under certain conditions, the fetus may also experience some oxygen deprivation. In women with limited ability to respond to increased oxygen demands during exercise due to cardiac or hematologic disease, the stress of exercise may be sufficient to jeopardize fetal health and development.

Specifically, oxygen consumption in a pregnant woman during exercise is greater than in the same woman after the birth of her child. Oxygen debt occurs when resynthesis of energy stores cannot keep pace with energy expenditure during increased muscular activity. The rapid breathing after exertion is the body's means of "repayment" of this oxygen debt.

The results of the study suggest that exercise, even when non-weightbearing, is more "oxygen expensive" during pregnancy in terms of maternal oxygen consumption and cardiac output. Increased muscular need for and consumption of oxygen can contribute to acid-base imbalance and deprive the fetus of oxygen. Significantly, evidence from diverse studies suggests that fetal growth is dependent upon the adequacy of maternal oxygen supply to the pregnant uterus. These observations emphasize the importance of understanding the physiological costs of exercise during pregnancy to help assure an optimum environment for fetal growth and development, particularly in handicapped mothers.

### Early Pregnancy Test for Primates

A group of NICHD intramural scientists have devised a simple, inexpensive test that will tell researchers whether or not a primate is pregnant as early as the 14th postcoital day. Dr. Gary

Hodgen and colleagues, as spin-off from their research on the structural, biological, and immunological properties of mammalian gonadotropins, have discovered how to test for chorionic gonadotropin in monkeys, baboons, chimpanzees, and marmosets approximately 2 weeks after coitus, which gives the investigator a definitive diagnosis of pregnancy during the week after implantation. This will considerably enhance a variety of research studies on teratogenicity and also on the etiology of birth anomalies, fetal development, and the efficacy of contraceptives.

It is especially important in teratogenicity studies to insure that agents are tested against the full span of fetal development. Early detection to date had been difficult. For example, one common method—rectal palpation—does not reliably detect pregnancy until nearly the 40th day of gestation. Another procedure is more sensitive but involves costly, tedious biologic assays of an animal's urine collected over a 24-hour period.

Chorionic gonadotropin is a hormone secreted by the placenta after implantation of the fertilized egg. Implantation occurs in primates from the 8th to the 10th postcoital day. While it was possible, by the bioassay method, to determine whether an animal was pregnant by analyzing urine collected on the 18th postcoital day, the urine had to be concentrated and detoxified and then injected into mice for 2 to 4 days. The results were not available for a week and, by this time, the animal was 25 days along in gestation.

The new test is based on the principle of hemagglutination inhibition (HI), a widely used immunoassay technique. The test reagents are lyophilized sheep red blood cells coated with human chorionic gonadotropin, an antiserum which agglutinates diluted suspensions of these labeled red blood cells, and a buffer solution used for diluting the cells. In performing the test, the presence of chorionic gonadotropin in urine specimens will inhibit the agglutination reaction, and the result is readily visible.

More sensitive than conventional bioassays, the test requires only a few drops (200 microliters) of urine and takes only 2 hours to complete. The procedure itself is inexpensive, and also eliminates the expense of housing the animals in order to collect 24-hour specimens. In addition, the assay is reliable and results in less than one percent of false negatives or false positives.

In the 1960's hundreds of children were born with congenital deformities to mothers who had used thalidomide in pregnancy. The effects of that drug on the developing fetus occur early in pregnancy, and tests then available were unable to detect pregnancy in primates early enough to screen for the drug's effects. By the time scientists could determine whether an animal was pregnant, the period of fetal vulnerability to thalidomide had passed.

Kits containing the reagents for the HI test for chorionic gonadotropin are being distributed by the Contraceptive Development Branch, Center for Population Research, NICHD, to scientists all over the world. Results thus far indicate that its reliability has held up under field conditions.



### Automated Chromosome Analysis

About 200 genetic diseases can now be diagnosed before birth: many of these diagnoses must be made by analyzing chromosomes for abnormalities. For example, Down's syndrome (mongolism) can be recognized in the affected fetus by identification of characteristic alterations in morphology or number of chromosomes. Karyotyping is the method of chromosome analysis. It is performed during the metaphase stage of cell division when chromosomes are spread out and more easily identified. As research in cytogenetics has expanded and antenatal diagnosis using amniocentesis has become more widely used, the demand for laboratory karyotyping has increased, but the high cost of the test limits its use. If the tedious labor involved in karyotyping can be reduced, the cost goes down, and the test can be applied much more widely.

The recent application of various "banding" techniques to permit detailed characterization of each chromosome has further pointed up the need for automatic karyotyping. It is difficult visually to discriminate, much less to quantify, minor chromosomal variations in size or changes in band widths or interband distances.

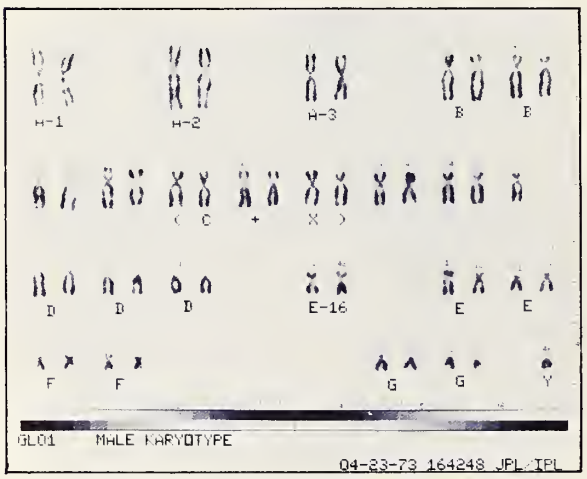
Under a contract supported by the NICHD and in cooperation with the National Aeronautics and Space Administration (NASA), computer scientists at the Jet Propulsion Laboratory of the California Institute of Technology have designed an automated system to karyotype both conventionally stained and "banded" chromosomes from human blood or other tissue samples. The system starts with a culture of blood, amniotic fluid, or other samples and produces karyotypes of selected cells.

The first step is semiautomated slide prepara-

tion, performed by a device developed by the City of Hope Medical Center in Duarte, California. This device can prepare almost 600 stained specimens a day.

The stained slides can be either stored for future use or proceed to the next step, at which a computer-controlled microscope searches for a suitable metaphase spread for analysis. Once a suitable spread is located, a scanning process digitizes the raw "scrambled" spread image, converts it to numerical form, and feeds it into the computer.






Karyotype print is produced in minutes by a computerized chromosome analysis system.

The computer then analyzes the digital image by locating, measuring, and classifying the individual chromosomes, and arranging their images into karyotype format. In the final step, the digital karyotype image is converted to a photograph by a special photographic recorder: the resulting karyotype print is the equivalent of its manually-produced counterpart in appearance and resolution, and can be interpreted in the conventional way.

One feature of the computer system which considerably enhances its efficiency is that it is possible to overlap the procedures of slide search, karyotype analysis, and photographic recording and allow them to proceed on three cells simultaneously. This cuts the processing time by two-thirds.

The system is designed so that an operator can intervene in its operations as circumstances require, and, at one point the geneticist must select cells for analysis. After the chromosomes from those cells have been isolated, they are displayed on a screen to allow the operator to evaluate and correct cases of chromosome touching or fragmentation. At another point, the karyotype is displayed to allow the operator to verify correct classification. After it is approved, it is sent to the photographic recorder, completing the process.

This computer chromosome analysis system represents a significant step toward moving automated chromosome analysis technology into the realm of clinical genetics and cytogenetic research.

### Oral Contraceptives and Thromboembolism

NICHD's Center for Population Research supports evaluation of contraceptive methods in use. An increased risk of thromboembolism in women using oral contraceptive steroids was recognized several years ago as a result of investigations conducted in Great Britain and the United States. Later evidence from surveys in Great Britain showed an apparent dose response relationship, suggesting that the risk of thromboembolism was greater in contraceptives having larger estrogen doses. (Estrogen constitutes an essential component of most oral contraceptives.) It was this observation which led drug regulating agencies of Great Britain and this country to recommend the use of contraceptives which contained less than 100 micrograms of estrogen.

An NICHD-supported epidemiologic study to evaluate the extent of these reported risks and to learn if the use of low-dose contraceptives did indeed reduce the risk of thromboembolism was recently completed by Dr. Paul Stolley and his associates of the School of Public Health, Johns Hopkins University. The study, begun in 1970, substantiates earlier results and provides a firm foundation for future investigations on oral contraceptives.

Participants in the study included women between the ages of 15 and 49 admitted to any of the 37 collaborating hospitals from 1970 to 1973 for thromboembolic disorders. Among these conditions were pulmonary embolism, thrombophlebitis of the lower extremities and other sites, venous embolism and thrombosis, arterial embolism and thrombosis, and acute myocardial infarction. Excluded were women who, at the time of their thrombotic complication, were not taking contraceptives.

Controls in the study were women admitted to the same hospitals for acute or chronic conditions and matched to the cases by race, age, and marital status.

It was found that the women with thromboem-

bolic complications, 78 percent had a history of prior thromboembolism, predisposing disease, recent surgery or trauma. This left 104 women—22.5 percent of the total—who had true idiopathic (cause unknown) cases of thromboembolism. When the relative risk of idiopathic thromboembolism was calculated it was found to be 7.2 times higher for oral contraceptive users than for women not using oral contraceptives.

Seventy-four cases and controls reported taking oral contraceptives containing less than 100 micrograms of estrogen per dose and 35 cases and controls took contraceptives with 100 micrograms per daily dose. More women with thromboembolic disorders reported taking oral contraceptives than did the controls in each dosage category. By comparing the proportions of contraceptive use between cases and controls, the scientists were able to determine the relative risk for each contraceptive formulation. They found that women using lower-dose contraceptives are 4.7 times more likely to develop thromboembolism than a non-user. The risk increases to 10.1 for users of contraceptives with the larger dose of estrogen.

The study demonstrated that the use of low-dose estrogen contraceptives substantially reduce the risk of thromboembolism. In fact, the users of high-dose estrogen contraceptives run almost double the risk of developing thromboembolism than low-dosage users.

### Role of Follicle Stimulating Hormone

The Center for Population Research also supports studies to expand our knowledge of mammalian reproductive processes. Much progress has been made recently in understanding the processes involved in sperm production. Investigators supported by the Institute have contributed to elucidating the role of follicle stimulating hormone (FSH) in the male reproductive process.

The pituitary hormones, lutenizing hormone (LH) and follicle-stimulating hormone, control reproductive function in men and women by regulating sex hormone synthesis, ovulation and spermatogenesis. An early hypothesis had been that LH controls androgen production and FSH sperm production. However, it is now believed that the functional differences between the two pituitary hormones are not that clear-cut. Androgen or LH *alone* can maintain spermatogenesis, although FSH is required to reinstate the process of spermatogenesis when the testes have atrophied following removal of the pituitary. In other words, *initiation* and *maintenance* of spermatogenesis may each have different hormonal requirements.

Two NICHD-supported research teams have recently identified a specific role for FSH in sperm production. The findings, reported by Dr. Frank S. French and his colleagues at the University of North Carolina, Chapel Hill, and subsequently confirmed by Drs. Barbara M. Sanborn and Anna Steinberger at the University of Texas Medical School, Houston, showed that one function of FSH in the testis is to stimulate the production of androgen binding protein (ABP) by the Sertoli cells. ABP functions as a carrier protein for male hormones and may facilitate accumulation of testosterone within the testes close to the developing sperm cells. The scientists speculate that FSH stimulation of ABP increases the concentration of androgen in the testes, thereby stimulating sperm production. The specific mechanism of androgen action on the maturing cells is not known. Further investigations by Dr. Sanborn have revealed that testosterone alone may be important in both the initiation and maintenance of Sertoli cell function.

Further support for the concept that Sertoli cells of the testes are targets for FSH has been provided by Drs. Allen W. Schuetz and John C. Davis of the Johns Hopkins School of Hygiene. Using special techniques for separating the cellular components of the testes, these investigators observed formation of colonies which appear to be composed of Sertoli cells when FSH is added to the culture; no colonies form following exposure of cells to human chorionic gonadotropins or serum bovine albumin.

Interference with the activity of androgen binding protein, or with its rate of synthesis could provide a means of selectively influencing the Sertoli cell response, and in turn, the regulation of spermatogenesis.

### Chimpanzee Aids in Linguistic Research

The development of language is as complex and subtle as the delicately interacting biological processes governing physical growth and development. Linguistic scientists have learned much from the study of children, but they have turned to primate models to probe how language emerges.

To understand better the conditions necessary for the emergence and refinement of various linguistic competencies, NICHD-supported investigators at Georgia State University, Yerkes

Primate Center of Emory University, the University of Georgia, and the Georgia Retardation Center have developed a computer-based training system to test a chimpanzee's "language" capability. A young female chimpanzee, Lana, was selected for their first subject.

Since the system has been in operation, Lana has learned to recognize by sight 90 "words" including the names of numerous objects and eight colors. She has learned to read those words, to complete sentences, to apply certain rules of sentence construction and to use



Lana at the computer console 'asking' for an apple.

prepositions. And she puts this knowledge to use without prompting, both to describe events and to request services of technicians in computer "conversations."

The training system consists of a PDP8-E computer, a variety of vending devices for incentives, and two keyboard consoles. The subject's console has 100 keys, each of which has a distinctive geometric figure—a lexigram—embossed on its surface. Each key, with its lexigram, functions as a word. As the locations of the keys are changed frequently, the subject must attend to the keys' lexigrams, not their positions, for successful use. For sentences to be formed, the keys must be pressed in sequences compatible with the grammar of the artificial language, *Yerkish,* devised for the project.

As the keys are depressed, likenesses of their lexigrams are produced from left to right in a row of projectors, which allows for message transmission and reception between subject and technician, and also for the subject to refer back to that which has been "said." Competency with the keyboard was gained through operant training procedures (rewards for correct responses or mild punishments for errors).

At first, Lana was taught simple key depression, then a number of "stock sentences" to gain a variety of incentives—food, liquid, a movie, music—which she could get whether her trainers were present or not. Shortly after, she started to use those sentences to solve novel problems and to formulate new sentences.

She can now ask that food be given to her, that things be moved from one place to another, and that food and drinks be put into her vending machines. Furthermore, she can improvise well enough to formulate names for new things.

According to the program's director, Dr. Duane M. Rumbaugh, and his associates, Lana's apparent transition into higher cognitive areas is much like the young child's handling of language complexities as it learns to speak. Therefore, as part of a recently approved NICHD project, Lana will remain the pilot subject in studies of increasingly difficult linguistic tasks.

A second program will assess the potential value of young apes as subjects for research into questions regarding initial language acquisition where ethical considerations bar use of the human child. A third endeavor will determine the feasibility of using the computer-based system to study language learning in the Georgia Retardation Center in Atlanta with children who have little or no expressive language competence.

Although research into language and language acquisition by young primates is still in its infancy, there is reason to believe that the fundamental processes involved are like those in man, and that a better understanding of these processes will be gained by studying the structural parallels between human speech and animal communication systems. By charting the unknown area between, scientists may be able to help children with impaired speech to communicate.

### Non-Speech Language for Handicapped Children

Teaching the profoundly retarded child to speak presents parents, psychologists, and speech pathologists with a prodigious challenge. These children, if not mute, can articulate only one or two words. Since verbal skill is critical to logical processes, problem-solving, and socialization, a nonverbal child is clearly at a disadvantage in interactions with others. Speech pathologists are beginning to explore, therefore, the usefulness to the retarded child of nonverbal speech.

For several years, much communication-related

research has been concerned with nonverbal language systems. Researchers have shown that retarded, nonverbal children can be taught the components of non-speech language, that is, that they can form associations between events and responses.

Dr. Joseph K. Carrier, Jr., of the NICHD-supported Kansas Center for Mental Retardation and Human Development, at the University of Kansas, has developed a program called Non-Speech Language Initiation Program, or Non-SLIP, which permits retarded, nonverbal children to work out simple sentences with the use of color-coded Masonite symbols. Some of these children even go on to vocalized speech.

Dr. Carrier teaches a speech-handicapped child to use color-coded symbols to form sentences.

The Non-SLIP system is based on the earlier work of David Premack, in which he devised a set of plastic symbols in various shapes to teach a young chimpanzee to communicate. The animal was taught to select the correct "words" and arrange the symbols sequentially on a magnetic response board as if writing a sentence. The symbols eliminated the need for learning spoken responses, and reduced the number needed by the communicator. The method appeared particularly promising for the retarded child since he would not be required to produce a distinctive sound for each word. To communicate, a child needed only to visually discriminate among the various shapes and place his choices on the board.

Dr. Carrier's approach distinguished between the classes of behavior a child must learn to perform according to linguistic rules, that is, to recognize various words and to develop sentence structure from among various syntactical (grammatical) arrangements. The child must be able to form associations among members of each of these sets and, finally, to use a symbol both expressively and receptively.

The training procedure requires first only that the child be able to discriminate among each of four different classes of stimuli: 1) set of six colors, 2)symbols cued to one strip of tape or two, 3) the shapes of Masonite symbols, and 4) pictures of people and animals in various actions. Most children were able to manage these tasks, but a few required training. Only two of more than 100 children trained were unable to do the tasks, but all, including a small number of youngsters classified as "deaf-blind," learned some basic discrimination skills.

In the next phase of the program, a child learns to arrange sequentially seven different forms on the response tray as if writing a simple declarative sentence of seven constituents. The parts of speech are tagged with color symbols; orange for nouns, blue for verbs, red for articles, and black for prepositions, the children in time learning simple grammatical structure as they see the symbols arranged in sentences.

The children are quick to learn associations between symbols and objects, so the symbols of nouns as concepts are introduced first. To encourage this, a correct response earns a reward. An incorrect response is ignored.

Once ten nouns are mastered, a child is introduced to verbs and the conceptualization of action, consistently the more difficult of the two symbols to grasp. When a child has some vocabulary in the four parts of speech, he learns how to select various constituents to make sentences.

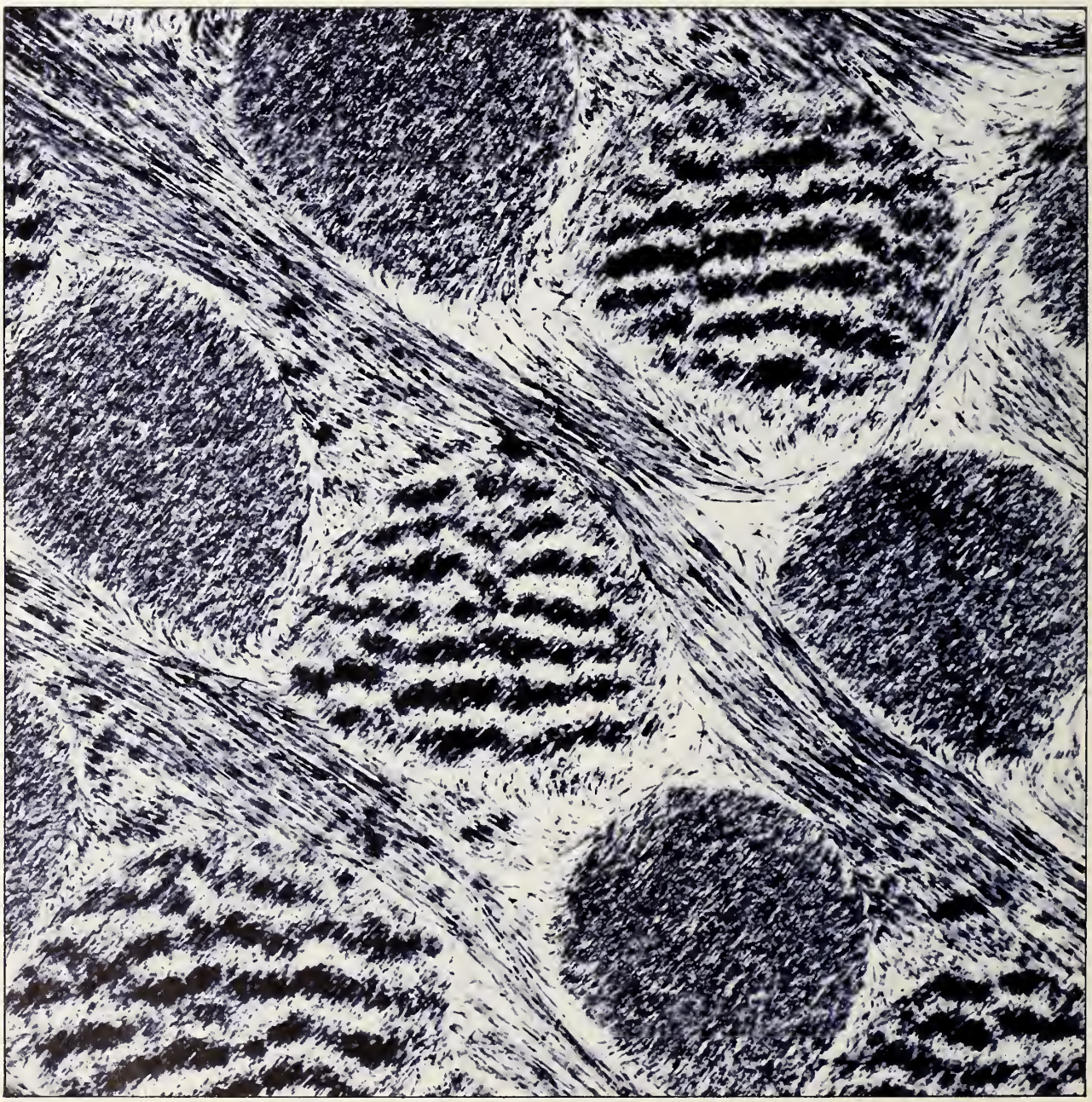
Toward the end of the program, some of the children attempt vocal imitations of the words and sentences they make and are ready for conventional speech therapy. Imitation has long been recognized as one of the pathways by which a normal child acquires speech.

Now more than 180 children are enrolled in Dr. Carrier's Non-SLIP program, all of them severely retarded. More than 95 percent have succeeded in acquiring some communication skills in the process. Some have progressed to speech clinics, and are able to communicate verbally. Others who have more severe deficits are still able to learn to use signs and gestures as a means of communicating.

Electron micrograph showing organization of crystals in rat enamel. Magnification X 13,000.

The hydroxyapatite crystals which form the enamel are organized into either long cylinder-like structures, called enamel rods, or inter-rod material which fills the space between the adjacent rods. The rows of circular structures are profiles of rods cut perpendicular to their long axes and the banding pattern in alternating rows is caused by sectioning.





### Transmission of Human Dental Infection

Animals reared free of germs do not develop tooth decay, but after infection with a decay causing organism such as *Streptococcus mutans*, decay follows. Thereafter the organism can be transmitted from animal to animal, and decay will occur.

*S. mutans* has been retrieved from decayed spots on human teeth, but little is known about how or when this microbe is transmitted to children. Since the organism has been found only in human beings and a few animals, the suspected method of transmission is human contact.

Streptococcus mutans colonies grown on mitis salivarius agar. Magnification X 48.

Aided by new techniques and culture media, a group of National Institute of Dental Research-supported scientists in Boston, Massachusetts, have found, that *S. mutans* rarely colonizes in babies' mouths until after the first teeth have erupted. The only exceptions among 101 infants without teeth were two who wore appliances constantly to close their palatal clefts. Eight others who wore the hard plastic coverings only for feeding had no *S. mutans* colonies before their teeth erupted.

Efforts to collect these organisms show how selective they are in their habitat. The investigators wiped the inside of each mouth with cotton-tipped swabs and, when appropriate, scraped the palatal appliances and the teeth. Mouth swabbing retrieved *S. mutans* from only one child who lost four upper front teeth to rampant decay at 18 months of age.

In contrast, scraping the tooth crown retrieved the organism from about half of the 17 babies whose teeth were scraped near the gum line. But when the part of the tooth crown most distant from the gum line was scraped, *S. mutans* was detected in only one infant.

A comparison of the types of *S. mutans* found in nine pairs of mothers and infants revealed that the c type was most prevalent among them as it is in the general population. One mother did have the rare *b* type. Several weeks later the scientists retrieved the *b* organism from her baby.

The finding suggests that children obtain decay-causing organisms from their mothers. The research also shows that *S. mutans* requires a hard surface in order to colonize successfully.

This study involved 138 infants from 3 weeks to 14 months of age. The collaborating scientists were Drs. Robert J. Berkowitz of Harvard University, Harold V. Jordan of the Forsyth Dental Center, and George White of Tufts University, Boston.

### "Green Light" for Adult Orthodontics

The recent finding that the upper jaw does not completely unite with the frontal bone of the skull until people are over 70 years of age makes it possible to treat patients with protrusion of the upper jaw (maxilla) and other related disfigurements well into adulthood.

The new knowledge, that final fusion occurs many decades later than anatomists thought it did, will encourage orthodontists to use traditional appliances instead of, or in addition to, surgery for treating midface problems.

Traditionally orthodontists reshape bone and move teeth by applying direct force to teeth, and thence to the supporting bone and sometimes joints. In time, pressure changes the shape of a tooth's bony socket and consequently the tooth moves to a new position. Sometimes the entire jaw should be moved to make a more harmonious face.

Vincent G. Kokich, D.D.S., and Benjamin C. Moffett, Ph.D., NIDR grantees at the University of Washington, Seattle, studied 61 skulls of humans aged 20 to 95 years, concentrating on the frontozygomatic sutures which lie on each side of the face where the zygomaxillary portion of the upper jaw meets the frontal bone of the skull. They examined the suture on one side by

Tracing of a section of the skull of a 95-year-old person showing incomplete fusion of the upper jaw with the frontal bone.



X-rays and close inspection. The other side, they compared histologically using a microscope to study stained sections of the tissues.

The investigators found that instead of fusing between the 18th and 35th years as expected, these bones did not unite until after age 70, and then only partially.

### Immunization Against Recurrent Herpes Infection

Recent experiments with viral immunization procedures show that it is possible to protect mice against persistent ganglionic infection caused by herpes simplex virus (HSV). Recurrent HSV infection is one of the most common viral afflictions of man. Even though the fever blisters caused by type 1 HSV are for the most part only a nuisance, this virus can cause severe eye damage as well as occasional encephalitis and death. The genital form of HSV, type 2, is the cause of one of the most common venereal diseases in the United States. Infants who contact this virus from their mothers often suffer from neurological disease or die from the infection. Moreover, there is some evidence that genital herpes may be linked to cervical cancer in women.

Yet, the factors responsible for recurrent herpes lesions on the lips, eyes, or genital areas, still remain a mystery to scientists. In investigating this virus, NIDR scientists tried to mimic the human disease by infecting mice in various sites such as lips, eyes, footpad, and genital regions. They found that the virus persisted in nerve cells (ganglia) connected to those skin surfaces which were originally infected. Several months after eye or lip infection, HSV was isolated from the trigeminal ganglia of the brain, and after footpad or vaginocervical infection, the virus was found in the dorsal root ganglia of the spinal cord.

Drs. Richard W. Price, M. Antoinette Walz, Charles Wohlenberg, and Abner L. Notkins then did further studies to see whether this persistent ganglionic infection could be prevented if mice were immunized before infection. They injected live virus into the peritoneal cavities of mice because this procedure produces antibody but does not cause a ganglionic infection. The next step was to challenge these animals with HSV by applying virus to selected skin or mucosal surfaces such as eye, lip, skin, vagina, and footpad.

The virologists found that 30 to 50 percent of unimmunized mice die when infected with type 1 virus via skin or vagina routes; however, none of the immunized animals died. Furthermore, from 70 to 95 percent of the immunized mice did not develop a ganglionic infection when the virus was applied to the cornea, lip, skin, or vagina. However, immunization did not prevent the development of a ganglionic infection when mice were challenged by the footpad route. Moreover, in this latter case, the scientists found virus in the ganglia of mice despite the fact that the immunized animals had high levels of antibody.

These studies demonstrated that prior immunization with HSV does provide some degree of protection. The investigators speculate that in those cases where there is mild tissue injury and little exudation of serum at the site of the infection, the virus is able to enter the nerve cell directly without interference from the host immune system. Once inside the cell, it is thought to be protected from antibody and can thus persist for months or even years.

Following original infection of mouse foot pads with herpes simplex virus, latent virus lives in nerves.

The investigators emphasize that much more research is needed before it can be determined whether a vaccine for human use would be of potential benefit in preventing HSV infection.

### Collagen Molecules Form Biological Rope

For many years scientists at the National Institute of Dental Research have studied a protein called collagen in order to learn the composition of its molecules and how they form rope-like fibers. The toughness, flexibility and binding qualities of these fibers account in large part for the properties of the matrix of bone and teeth, the meshwork that holds teeth in their bony sockets, the wear-resistant texture of cartilage, the tensile strength of tendons, the resiliency of blood vessels, and the durability of skin. In fact, 20 percent of the protein in higher animals consists of collagen.

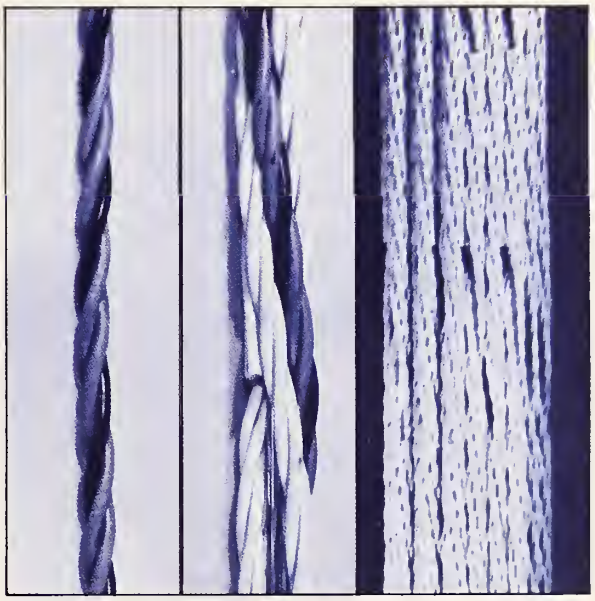
Institute scientists have learned that there are at least three different collagen molecules in humans, each under the control of separate genes. Type I is the most common and best known; type II occurs in cartilage but not elsewhere; type III predominates in blood vessels and intestine. They have different rates of turnover, chemical properties, and presumably characteristic differences in function.

However, all collagen molecules are basically similar and consist of three long chains of amino acids. For most of their length, the chains coil around each other to form a triple helix. The most studied chain, called the alpha one chain, has 1052 amino acid units or residues of which 16 at one end and 25 at the other are not in the helix but are utilized for binding and crosslinking to other collagen molecules. The helical portion (1011 residues) is remarkably uniform in that each chain consists of 337 triplets of amino acids, one of which is always glycine. Type I collagen contains two of the alpha one chains and another very similar chain called alpha two. The collagen molecule is about 30 nm long and 1.4 nm wide. A nanometer (nm) is one billionth of a meter.

Collagen molecules associate to form thread-like microfibrils, which are regular helices of molecules 40 nm wide and many microns long. These in turn coil together to make larger rope-like fibrils which sometimes reach widths of several hundred nanometers. Parallel arrays of fibrils, such as the fibers in tendon, may be several millimeters or more in diameter and have the tensile strength of a steel cable. It has required a combination of biochemical, X-ray diffraction, optical diffraction and electron microscopic techniques and computer analysis to establish how these structures are formed and what holds them together.

The evidence suggests that collagen molecules self-assemble in a highly specific way, first to form the microfibrils and then larger structures. How this is regulated biologically is presently being studied. Once assembled, covalent cross-links form between chains and molecules to impart strength to the final product.





(l. to r.) Model of a 3-chain collagen molecule; a 5-molecule collagen microfibril; and a 36-molecule collagen fibril

Pathological changes can result from heritable defects that alter the structure of collagen or affect its crosslinking. The molecular basis of several such diseases has been established. Other diseases, most notably those involving inflammation and age changes, also affect collagen by degradation or by more subtle changes. These processes are not well understood, but knowledge about collagen chemistry and structure has stimulated many biomedical studies on these problems.

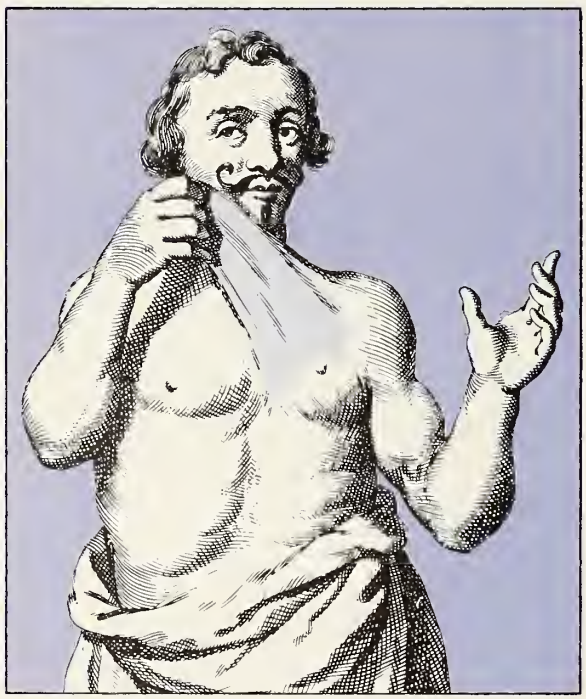
### Exploring Causes of Collagen Diseases

By carefully analyzing some inherited human diseases, scientists at the NIDR, Johns Hopkins University, and elsewhere have obtained new information about the formation of the strong connective tissues necessary for normal body function. Their research shows how essential to health are various cooperating mechanisms for linking and assembling collagen molecules.

One illuminating disorder is the Ehlers-Danlos Syndrome, of which there are at least seven variants. The first three forms, of unknown cause, are dominantly inherited. Patients with these as well as most other forms have stretchable skin and joints and bruise easily.

Patients with type IV Ehlers-Danlos Syndrome have thin but not stretchable skin. They bruise readily and are liable to catastrophic ruptures of large arteries and intestines. Drs. George R. Martin, NIDR, and Victor A. McKusick, Johns Hopkins, identified this disorder as a deficiency of type III collagen which is an important constituent of skin, intestines, and arteries. Its lack weakens these tissues.





In 1657, professors at the Academy of Leyden examined a patient who demonstrated the stretchable skin of one form of the Ehlers-Danlos collagen disease.

Patients with type V, VI, and VII of the Ehlers-Danlos Syndrome have defects that interfere with the crosslinking of collagen. The investigators found that type V patients have too little lysyl oxidase, the enzyme that crosslinks collagen molecules together.

Type VI patients suffer from severe scoliosis (twisted back) and are susceptible to irreparable eye damage from detached retinas. Their collagen lacks hydroxylysine, an amino acid used in crosslinking. This defect was established by scientists at the Massachusetts General Hospital and confirmed in these studies.

Because of dislocated joints, type VII patients are short. They are also liable to have loose skin and to bruise easily. Drs. Martin and McKusick find that these people accumulate a large precursor molecule instead of converting it into normal collagen. The precursor distorts and weakens collagen fibers because it cannot assemble properly into normal "biological rope."

With the exception of type IV, all patients with the known variants of Ehlers-Danlos Syndrome have abnormal crosslinking which weakens the collagen in their tissues.

The scientists also studied osteogenesis imperfecta in collaboration with Dr. Jack R. Lichtenstein of the Armed Forces Institute of Pathology. This is another heritable disorder which produces weak skeletal tissues liable to fractures. Patients with severe forms of the disease may die at birth while others may lead normally active lives. The investigators learned that some severely afflicted patients do not synthesize type I collagen, the major protein constituent of mature bones. Patients with less severe forms of this disorder may have other defects that reduce the amount of type I collagen deposited in the bones which therefore lack strength.

Fortunately, genetic collagen diseases are relatively rare because most of the abnormal genes which cause them are recessive and cause serious problems only when inherited from both parents. Scientists believe that each protein, including every enzyme, is under the control of at least one gene. Therefore, a variety of defective genes must be involved to prevent the synthesis of a type of collagen, to hinder crosslinking, and to interfere with collagen's assembly into fibrils.

### Complement Induced Bone Destruction

Drs. Ann L. Sandberg and Stephan E. Mergenhagen of NIDR in collaboration with Dr. Lawrence G. Raisz of the University of Connecticut, Farmington, and Dr. Jo Max Goodson of the University of California, San Francisco, have demonstrated a new biological function of complement, that is, bone destruction. The complement system is a series of serum proteins which act in sequence. Activation of this system produces such diverse immunologic effects as cellular destruction, the attraction of inflammatory cells, enhanced engulfment of bacteria and cell debris, increased permeability of blood vessels, and viral neutralization.

Now the scientists say bone loss in such pathological conditions as periodontal disease

and rheumatoid arthritis may be attributed, at least in part, to activation of complement. After antibody interacts with antigens, either present on cells or possibly adherent to cell surfaces (bacterial products, etc.), complement is activated locally and bone resorption follows.

The investigators detected this resorptive effect of complement in cultures of fetal rat bones. They injected pregnant rats with radioactive calcium which was incorporated into the bones of the fetuses. Next the scientists cultured the fetal bones and measured the bone destruction

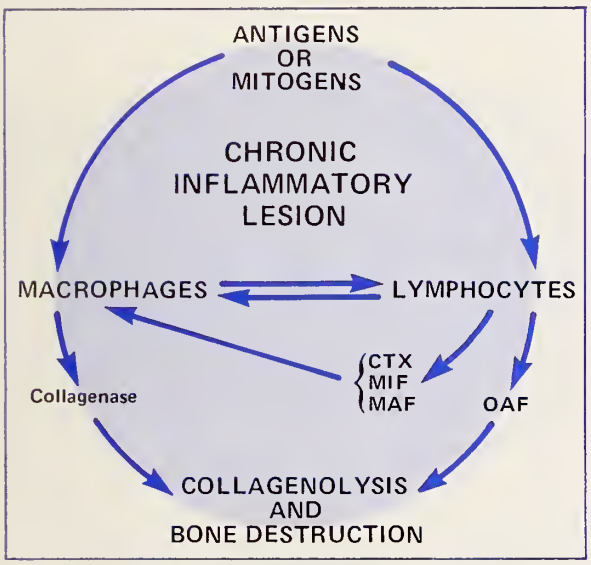



Diagram of immune factors triggered by bacterial products that can destroy bone.

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by determining the release of radioactivity into the culture fluid. When they added antibodies specific for cell-surface antigens together with complement to the culture medium, a marked increase in the release of calcium occurred.

Activation of either the classical complement pathway or the more recently described alternate pathway started this loss of calcium. However, bone resorption did not occur if the complement was destroyed by heating or if the complement source lacked a late complement component (C6). In the later case, resorptive activity could be restored by adding this component to the deficient complement. At the termination of culture, microscopic examination revealed that resorption caused by complement was similar to that observed when bones were treated with the osteoclast activating factor derived from white blood cells.

The investigators established that complement-induced bone resorption is mediated by prostaglandin E, a potent resorbing agent which has been detected in high concentrations in inflamed joint and periodontal tissues. Prostaglandin E is a small, biologically active molecule that is synthesized in many tissues. The scientists demonstrated prostaglandin involvement in this reaction by showing that Indomethacin, an inhibitor of prostaglandin synthesis, blocked the resorptive effect. In addition, high levels of prostaglandin E were detected in the media of cultures which contained specific antibodies and complement.

Thus immune activation of complement at a cell surface stimulates synthesis of prostaglandin which in turn destroys the adjacent bone.



## Cell Mediated Destruction of Connective Tissue

In addition to the destruction of bone and collagen that can be caused by reactions of complement in the blood, there are at least two other mechanisms by which white blood cells can damage tissue. These immunologic factors have been suspected for several years as contributors to the loss of bone found in periodontal disease. There is also considerable evidence that the same mechanisms affect joint tissues in rheumatoid arthritis. According to Drs. Stephan E. Mergenhagen, Sharon M. Wahl, and Larry M. Wahl of the NIDR, and John E. Horton of the U. S. Army Institute of Dental Research, and Lawrence G. Raisz of the University of Connecticut, Farmington, in both of these diseases, leukocytes migrate into inflamed tissues and destroy bone and connective tissue.

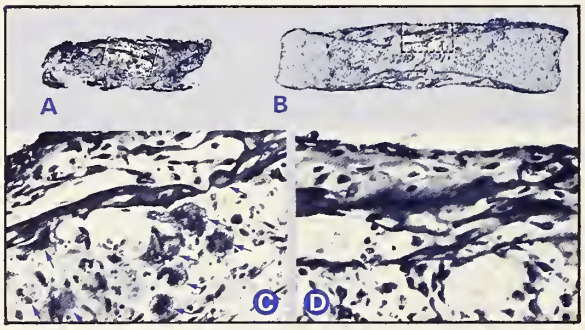
The investigators report that two types of white cells—lymphocytes and macrophages—act in conjunction to damage bone and collagen. In blood vessels, macrophages normally scavenge dead cells and other wastes, but the scientists found that under the stimulus of chronic immune reactions, these cells will congregate in inflamed tissue and there secrete collagenase, an enzyme that destroys collagen and the organic part of bone.

The scientists found that unstimulated guinea pig macrophages made no collagenase when cultured by themselves. Neither did they make the enzyme when cultured with unstimulated lymphocytes from guinea pig spleens. However, the macrophages produced collagenase when incubated with culture fluids removed from stimulated lymphocytes. These fluids, which were obtained from cells cultured with and stimulated by an antigen derived from human dental plaque, contain lymphokines. Lympho-

kines are biologically active molecules which take part in many immune reactions such as attracting white cells to an infected area, preventing macrophages from migrating away from an inflamed region, or killing specific cells.

These immunologists also discovered a new lymphokine which stimulates resorption of bone and have termed it "osteoclast activating factor" (OAF). They learned that OAF appears in cultures of human lymphocytes only after they have been stimulated by either a specific antigen






(a) Rat femur resorbed by OAF. (b) Another femur growing normally. (c) Arrows show osteoclasts assimilating bone. (d) normal bone without osteoclasts.

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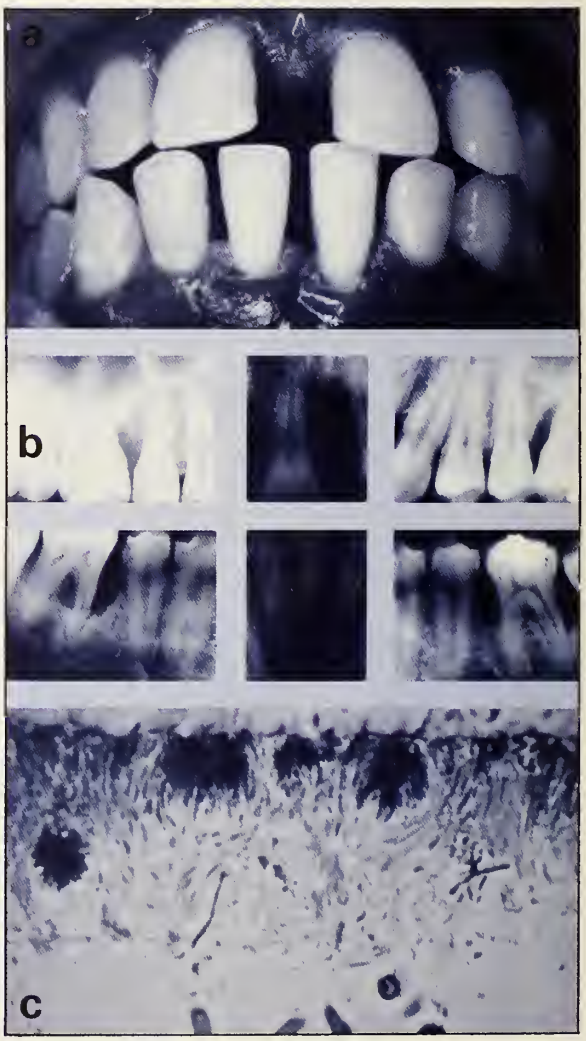
or a nonspecific mitogen, a substance which triggers cell division. But once lymphokine is released, it stimulates cells called osteoclasts to destroy bone. Antigens, mitogens, or unstimulated lymphocytes alone produce no OAF. Even stimulated lymphocytes do not make OAF without the contact with a few macrophages. The immunologists found that a ratio of only one macrophage to 40 lymphocytes was sufficient for OAF production, but the reaction did not take place unless there was direct contact between the two types of cell. Incubation of one cell with culture medium from the other yielded no OAF.

This research indicates that collagenase and OAF are made in response to a variety of specific immune challenges, and suggests that much of the bone and collagen destruction found in wasting diseases is probably caused by immunologic reactions of specific cells to chronic exposure to bacterial or other antigens.

### Periodontosis May Be Controllable

New evidence implicates unusual bacteria in a dental disease called periodontosis and indicates that antibiotic control may be effective. This uncommon but severe type of periodontal disease strikes people between ages 13 and 25. Unlike periodontitis, the more common type of slowly-progressing periodontal disease, periodontosis destroys tooth-supporting alveolar bone rapidly. Teeth loosen and become useless with almost no warning, little inflammation, little plaque, and no pain. Since knowledge of the cause has been sparse, treatment has been difficult.

Encouraging reports have recently come from the Forsyth Dental Center and Harvard University in Boston, Mass., where NIDR-supported scientists have recovered specific organisms from patients with the disease and have shown that these same bacteria will produce a similar disease when implanted in germ-free rats. Furthermore, the scientists have been able to control the disease in patients with antibiotics.




(a) Mouth of a teenager with periodontosis has a normal appearance. (b) Radiograph of same teenager shows severe bone loss around front teeth and early loss around first molars. (c) Tooth root (top) covered with sparse bacterial plaque shows how rod bacteria sensitive to oxygen grow.

Although little inflammation or dental plaque (accumulation of organisms) is seen in periodontosis, the investigators nevertheless believe that microbes found deep in the pockets around the affected teeth might contribute to the problem. To isolate the bacteria from the diseased sites, the microbiologists used new techniques they had developed that enable organisms sensitive to air to survive in culture. Their results showed that five groups of unusual Gram negative, rod-shaped anaerobes (organisms that grow only in air-free environments) predominated in the highly localized sites of periodontal destruction.

When each of the five types of these rod-shaped organisms taken from patients with periodontosis was implanted separately in germ-free rats, the animals developed severe loss of alveolar bone within 42 days although no plaque accumulated. By 84 days, the periodontium was virtually destroyed, according to Drs. James T. Irving, Michael G. Newman, Sigmund Socransky, and Mr. John D. Healey.

Later, Drs. Socransky and Newman and associates studied families of patients with periodontosis and found that similar groups of organisms could be recovered both from family members with the disease and from younger children without the disorder.

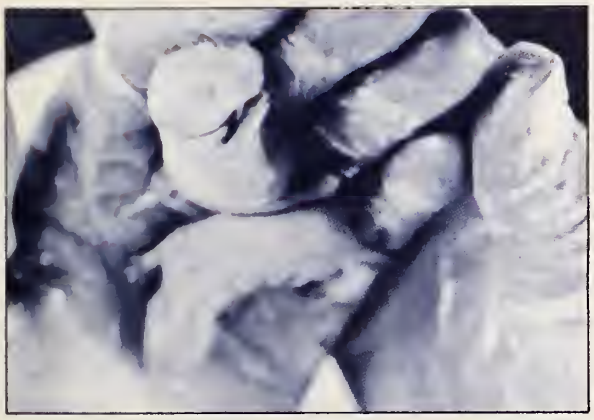
Because these bacteria were found in diseased sites, and because they could produce disease in animals, the investigators began limited tests of antibiotics in patients. Already, preliminary reports show that tetracycline can control the disease in a few carefully-monitored patients.

NIEHS attempts to develop methods for testing the thousands of man-made chemicals already in our environment, as well as the hundreds of new ones introduced each year, that have never been tested for toxicity.



## Test Systems for Birth Defects

Probably no health-related disorders are as tragic as those resulting in malformed babies. To help prevent these tragedies scientists at the National Institute of Environmental Health Sciences are developing models to study the mechanisms by which teratogenic effects (birth defects) are produced and are seeking animal models to rapidly predict human teratogens. These models include mouse, rat, and rabbit embryos and mouse cell, tissue, and organ culture systems.



NIEHS researchers are seeking animal models to rapidly predict which environmental agents may cause birth defects in humans.

Drs. Robert E. Staples and Ralph R. Maurer are determining the potential of direct application of test agents to mouse, rat, and rabbit embryos for detecting teratogens. Direct exposure circumvents changes in the compounds induced by the maternal drug processing system and permits more accurate determination of the amount of test compounds to which the embryo was exposed. Embryos are taken from donor animals and then exposed to various chemicals or conditions during culture. Following exposure some embryos are reimplanted into the uterus of females; others are examined for structural or chemical changes. Near term the re-implanted embryos are scored for defects. Results are compared to those seen following administration of the test agent to the dam.

This procedure is being validated with well-studied chemicals such as thalidomide, a drug which resulted in hundreds of babies with malformed limbs born to women who, when pregnant, took it as a tranquilizer. Accumulated data indicate that high thalidomide doses are lethal to the embryo but do not affect formation or development of blastocysts (an early stage of embryo development). To date structural malformations have not resulted from direct application of thalidomide or additional teratogens to the embryo.

Viable mouse tissue and organ culture systems are being developed by Dr. Judson W. Spalding and his co-workers to investigate the mechanism by which chemical agents affect development of germ cells (reproduction cells). These cells—if defective—often result in sterile offspring or offspring with many inborn genetic errors.

Various environmental agents and drugs are known to affect reproduction and development, but their specific molecular effects on germ cells (oocytes and spermatocytes) during the early stages of development are not well understood. In ovary organ systems, Dr. Spalding has shown that oocyte maturation occurs in a manner analogous to oocyte maturation in the whole animal *(in vivo)* to about 25 days postconception. Continuing work is aimed at obtaining consistent and reproducible stages of growth and maturation. When this model is fully developed effects of chemicals on embryonic and postnatal germ cell differentiation may be compared with effects on the development of the male and female sex cells (gametes) in the whole animal.

## Short-Term Tests for Mutagenic Activity

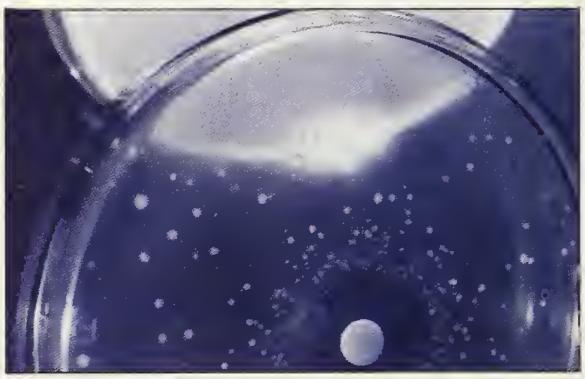
Discovery of cancer among workers exposed to vinyl chloride (a gas from which polyvinyl chloride is made) emphasizes the need for better ways to evaluate other potential hazards in the environment prior to man's exposure. These hazards include mutagenic agents, or agents that cause genetic damage. The damage may take such forms as chromosome alterations that can cause spontaneous abortions or an increase in inheritable diseases such as hemophilia.

Development of short-term qualitative tests to evaluate the thousands of man-made chemicals already in the environment that have never before been tested for mutagenic activity is the aim of Dr. Frederick J. de Serres and his co-workers at NIEHS. They have made progress in developing *in vitro* or test tube techniques for metabolic activation. Now mammalian metabolites, often the active mutagens, can be tested, as well as the original compound. Because of this the use of whole animals can be reserved for obtaining quantitative data required to confirm and evaluate risk.

These short-term tests are widely regarded as a sensitive initial method of quickly and cheaply

screening chemicals and consumer products for possible toxic effects and of singling out suspect compounds for more detailed evaluation in higher organisms. Exploratory testing is turning up new examples of genetic activity in products in widespread use. For example, almost all commercially-available hair dyes recently were discovered to be genetically active in the newly-developed short-term tests. The potential hazard for the estimated 20 million people in this country who dye their hair is not yet known. NIEHS has begun studies to determine whether





Short-term tests for mutagenic activity may provide a testing capability for detection of adverse toxicological effects that can be used in early phases of product development.

hair dyes cause genetic damage in experimental organisms in order to better evaluate the risk for human beings.

These tests may offer answers in another area of concern. Although the relationship between mutagenicity and carcinogenicity (cancer-causing ability) is still a matter of considerable scientific controversy, contract work—support by the National Cancer Institute and developed with NIEHS assistance—is clearly showing a high correlation between the two types of activity. Thus the newly-developed short-term tests with microbes in combination with *in vitro* metabolic activation techniques appear to offer a means of detecting potential mutagenic and carcinogenic effects in man.

The tests may also be able to be used by industry to detect possible adverse toxicological effects in the early phases of product development. Industries have often invested considerable time and resources in a product only to find that it is carcinogenic after completing the traditional 2-year assay on laboratory animals. The short-term tests for mutagenicity offer a rapid, inexpensive mechanism for weeding out products with potential adverse effects early in the development process.

The compound AF-2, a nitrofuran derivative widely used as a food preservative in Japan beginning in 1965, exemplifies the potential of this approach. AF-2 was first determined to be mutagenic in the newly-developed short-term tests over a year ago. Much later it was found to be carcinogenic in mice. As a result AF-2 now has been eliminated from food preservatives in Japan. Vinyl chloride was also found to be genetically-active in these tests, but long after industrial workers and the population in general were exposed to vinyl chloride.

This emphasizes the need to develop better methods for determining effects of exposure to mutagenic chemicals on man. In addition, compounds structurally similar to vinyl chloride must be studied to learn if they are genetically active, and if so, to evaluate the effects of exposure. NIEHS investigators are now studying vinyl bromide, a compound structurally similar to vinyl chloride, which is used to make flame resistant fabric. Because this fabric can save lives it is important to determine whether vinyl bromide may be highly toxic and, therefore, unsafe to use without careful containment.

The difficulty with the discovery that AF-2, for example, is a potential mutagen and carcinogen is our inability to evaluate the immediate and long-term effects directly on man. Although the genetic basis of many human diseases is well established, scientists are extremely limited in their ability to determine the immediate effects of genetic damage. And the long-term effects, such as an increase in cancer incidence, would not be expected to appear for at least 15 to 20 years. This may produce an illusion of safety based on inability to test rather than on sound scientific data. For this reason NIEHS scientists are giving high priority to the development of meaningful tests on human beings a short time after exposure to environmental compounds in order to determine and evaluate the effects of such exposure.

### Estimating Human Risk of Carcinogens

As previously noted, the search for causative agents of such diseases as cancer and genetic disorders must begin long before clinical manifestations begin to appear. Because the latency period (time from initial exposure to effect) associated with these diseases often ranges from 10 years to two or more generations, a great many people may be exposed to irreversible deleterious health effects if we wait for sufficient human data to accumulate to conduct an assessment of risk.

Thus decisions regarding acceptable levels of various environmental agents are being based on information derived from animal experimentation, where cancer latency periods are reduced to intervals of 12 to 18 months and entire lifetimes span 2 to 3 years.

The procedure generally employed to estimate risk associated with human exposure to a potential environmental carcinogen usually begins with the determination of the carcinogenicity of the specified agent at relatively high doses in laboratory animals. The observed risk is then extrapolated or projected down to human exposure levels and equated to human risk by some conversion factor.

One of the major obstacles in using animals for predicting human response is the problem of extrapolating to low dose levels, a procedure that involves use of a mathematical model of the carcinogenic process. Because two or more models may nearly coincide in the experimental dose range but yield widely divergent results in the actual range of human exposure, the model chosen for extrapolation is critical in determining the order of magnitude of the resulting estimate of the human cancer risk.

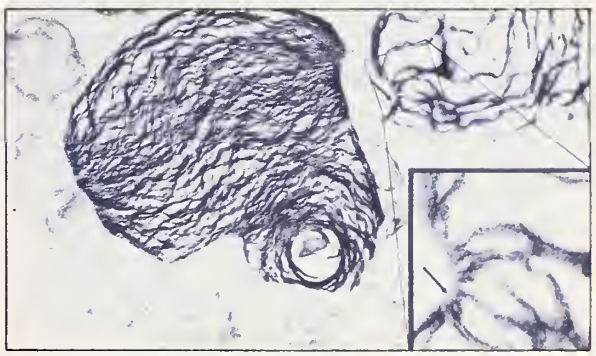
Recently a team of NIEHS researchers headed by Dr. David G. Hoel has been investigating various probablistic and mathematical models for extrapolation with particular emphasis on models that specify possible biological mechanisms of carcinogenesis. They evaluated the importance of background incidence and tumor induction in risk estimation. This evaluation indicated that the manner in which background is incorporated into a particular model has a pronounced effect on the biological interpretation of the mechanistic process or carcinogenesis, as well as on the estimate of the level of cancer risk to which the human population will be exposed. In addition, it was found that if carcinogenesis by an external agent is considered to be augmenting an already ongoing spontaneous process then a wide class of models predict that the response to the agent will be linear at low doses.

These findings have been used to develop guidelines for selection of sample size and dose levels in animal carcinogenesis experiments. One of the design principles delineated in this effort indicates that increasing the number of animals beyond a certain level does not appear to elicit a proportional increase in benefit as measured in terms of test sensitivity. Finally procedures for selecting optimal experimental dose ranges have been investigated. Work will continue on these methods so that the potential risk from exposure to particular environmental agents can be more realistically evaluated.

## Pulmonary Secretions

Very little is known about the diverse mixture of materials the lung secretes into its airways. At least some of these materials are vital for normal lung function. For example, without the surface-tension-lowering material called surfactant covering the air sacs (alveoli) of the lung, the lung would collapse. This surfactant, apparently lacking or deficient in premature infants afflicted with respiratory distress syndrome, appears to be secreted by cells located in the alveoli. Abnormal or excessive secretions can present problems. For example, people with cystic fibrosis, bronchial asthma, and alveolar proteinosis, as well as some occupationally-related pulmonary disorders, suffer from airways clogged by such secretions.





Material which accumulates in alveoli of patients suffering from alveolar proteinosis appears to derive from subcellular structures secreted by the alveolar Type II cells

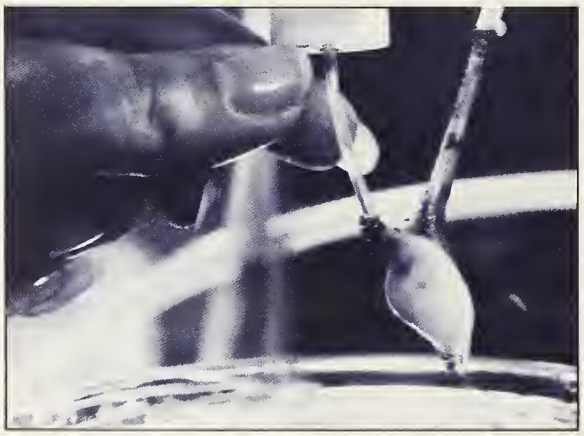
Studies by NIEHS investigators Drs. Gary E. R. Hook, Richard Mirel, and Richard P. DiAugustine, in collaboration with scientists at Duke University, indicate the presence of certain hydrolytic enzymes, lung-specific glycoproteins, and phospholipids in pulmonary secretions. The composition of these secretions correlates with specific disease states. For example, the material which accumulates in the alveoli of patients suffering from alveolar proteinosis appears to derive from subcellular structures secreted by the alveolar Type II cells. This indicates that in this disease there may be a malfunction of a specific epithelial cell.

Although the functions of most of these components in the airways of the lung are not yet known, their presence opens completely new avenues for diagnosis and early detection of various pulmonary diseases.

### Toxication-Detoxication in the Lung

The first stage of processing several groups of chemicals, drugs, and environmental pollutants by the body results in formation of substances called metabolites more toxic to mammalian systems than the original environmental contaminant. This process is referred to as "toxication." Some of these metabolites then may be detoxified—or made less harmful—by other biochemical systems in the body if these are present and not depleted by some nutritional deficiency, exposure to other chemicals or drugs, or genetic defect.





An isolated perfused lung technique used by NIEHS scientists permits them to study rat and rabbit lungs outside the body while the organs are functioning normally for limited time periods. Thus, these investigators can make certain that the effects measured are all happening in the lung.

Because the lung is the primary site of chemical toxicity the processes of toxication and detoxication in the lung may play a significant role in the overall toxicity of chemicals. In an attempt to understand these processes in the lung, NIEHS researchers Drs. Zvi Ben-Zvi, F.C.P. Law, and John R. Bend are studying how the lung metabolizes or processes a model compound, styrene oxide. Styrene oxide is a metabolite formed from styrene, one of the most widely used chemicals in the polymer industry. It is an excellent and relatively safe-to-use compound suitable for investigating the metabolism of more toxic chemicals, such as the polycyclic aromatic hydrocarbons, pollutants known to cause cancer.

An isolated perfused lung technique improved upon at NIEHS is being used to assess the mechanisms of styrene oxide effects on the lung. This technique permits scientists to study rat and rabbit lungs outside the body while these organs are functioning normally for limited time periods. It keeps the lung "alive" in the sense that it operates as it would in the body. Because blood carries foreign substances both from and to the lung, and some substances are detoxified in other organs and tissues, it is necessary to know how particular compounds get into the lung and how the lung alone handles them. The system permits investigators to make certain that effects measured are all happening in the lung so they can understand factors involved in the lung's formation and degradation of metabolites.

In their studies with styrene oxide the NIEHS group is attempting to compare the whole lung metabolism of styrene oxide to data from *in vitro* studies performed earlier. Results show that a major metabolite of styrene oxide in the perfused lung is a conjugate of the compound with glutathione, a naturally-occurring substance in the body capable of conjugating and thus detoxifying metabolites.

Glutathione levels in the lung may be the limiting factor in detoxication of styrene oxide. As glutathione levels in the lung decreased during the lung's continuing exposure to styrene oxide, edema or swelling was noted. If these levels are the limiting factor then it may be possible to protect workers exposed to styrene oxide by adding supplementary compounds, which are glutathione building blocks, to their diets.

### How Marine Species Handle Pollutants

Other NIEHS scientists are using marine species as models for studying human functions and diseases. Marine models are valuable because the simplicity of their structural or metabolic specialization permits study of a single problem at a time.

These organisms possess a vast repertoire of simple adaptations that make them especially suitable for studying human metabolism and the great diversity of marine organisms in our aquatic environment offers a vast potential for such studies. Moreover, marine species are often particularly sensitive to pollutants.

Finally, the home of marine species, the sea, is the ultimate receptacle for most environmental pollutants. Once pollutants enter the marine environment they frequently are taken up by marine organisms and can enter the food chain at highly concentrated levels. Because many of these organisms are important components of the human diet we must know how marine life handles and stores various pollutants in order to prevent human exposure.

Drs. Margaret James and John R. Bend are studying how and why marine species, especially edible marine organisms, accumulate pollutants. They hope to document how metabolic pathways (the processes the body uses in handling substances) differ in various species because this difference may determine how long environmental contaminants persist in many marine species. This could be an important factor in determining which species are safe for human consumption.

Some of their work has focused on the metabolism of the widely used herbicide 2,4-D (2,4-

Marine species are valuable as models for studying human functions and diseases because the simplicity of their structural or metabolic specialization permits study of a single problem at a time.

dichlorophenoxyacetic acid) in various marine species including the dogfish shark. The shark makes an excellent model since it has a simpler, but essentially similar, metabolic factory to man's. The liver of the shark, while architecturally similar to that of man, appears to perform more of a storage than a metabolic processing function. Some of the biosynthetic activities carried out in the enormously complex human liver are undertaken by the stomach and intestine in the shark. Thus, the shark liver offers a somewhat less complex chemical laboratory for study. Nevertheless, the biochemical transformations that take place in this organ seem to be comparable to those that occur in the human liver.

In their studies with 2,4-D, a contaminant of the aquatic environment and a member of a class of compounds which show considerable species variability in metabolism, the NIEHS investigators found that there is a qualitative difference between fish and mammals in the metabolic pathway of certain carboxyl compounds known to exist as environmental pollutants.

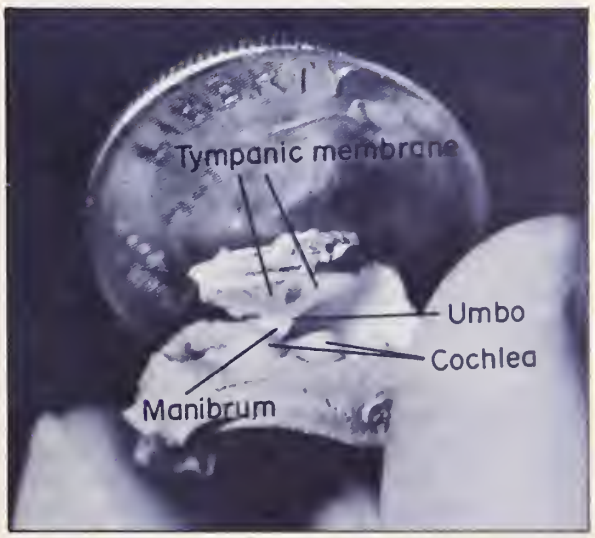
After 2,4-D was administered to dogfish shark, about 70 percent of the 2,4-D could be recovered from urine collected by catheters after 48 hours. Drs. James and Bend found that 2,4-D is excreted much more slowly by fish than mammals. Most of the recovered 2,4-D (more than 80 percent) was metabolized to a metabolite not normally produced by humans. This metabolite showed characteristics of a sulfonic acid, later identified as the conjugate of 2,4-D with taurine, an amino acid.

### Device Offers Unexpected Benefits



While devising techniques to evaluate how the ear handles acoustical data, Drs. Reginald O. Cook and Teruzo Konishi developed, and defined the parameters of, a device to impart complex signals—including speech—to the cochlea, the essential organ of hearing. This device may have important implications for people with certain kinds of correctible hearing loss.

Conventional hearing aids are poor fidelity devices. In the necessarily small hearing aid, a tiny acoustical diaphragm must be used to generate sound, a difficult mechanical-to-acoustic step. A small diaphragm simply cannot perform acceptably over a wide amplitude and a wide range of frequencies. For efficient production of sound, particularly in the low frequencies, a fairly large vibrating object is needed. It has also been difficult to produce high-intensity, wide-frequency sound from small acoustic sources without producing significant distortion at the same time. Finally, there are problems of feedback and squeal when the microphone and speaker are placed close together.




View of guinea pig middle ear. Most of bulla has been removed

Otologists, physicians who specialize in ear problems, have long thought that implanted devices might be capable of imparting speech directly to the inner ear by driving the ossicular chain of small bones of the middle ear. It was postulated that this could be done by a tiny direct-contact transducer because the difficult mechanical-to-acoustic step would be bypassed. But until now no one had extensively investigated the fidelity which might be possible by using such devices.



Now Drs. Cook and Konishi have developed a tiny piezoelectric transducer which when in direct contact with the ossicular chain can impart highly defined signals into the cochlea. This device will be used at NIEHS to deliver precisely defined signals to the inner ears of research animals.

### Emphysema Process Depicted

Studies underway virtually since the creation of NIEHS in 1966 have uncovered strong evidence that two pollutants in our environment—ozone and nitrogen dioxide—are contributing if not primary causes of pulmonary emphysema and chronic nonspecific pulmonary disease. By providing a clear picture of the sites and mechanisms of ozone and nitrogen dioxide toxicity and an understanding of pathologic events and structures involved, the NIEHS studies have suggested clinical approaches that might be useful in preventing and treating lung damage induced by these pollutants. Deaths from pulmonary emphysema have increased since 1950. And, chronic nonspecific pulmonary disease is now a leading cause of disability among adult males.

A part of the picture establishing the sequence of events of ozone and nitrogen dioxide toxicity was provided by Drs. Gustave Freeman, Robert Stephens, and their colleagues at Stanford Research Institute who produced an experimental facsimile of human emphysema in rats. In their work intermittent exposure of the animals for a lifetime (about 2½ years) to 15 parts per million (ppm) nitrogen dioxide ($NO^2$) caused emphysema and a chronic obstructive lung disease. There was a loss of about two-thirds of the air sacs (alveoli) with an attendant loss of oxygen-absorbing capacity.

Ozone was shown to be approximately 20 times more toxic than $NO^2$. It induces a similar type of disease but with certain significant differences. The initial injury is at a slightly deeper position in the lungs, and connective tissue growth at the sites of injury is stimulated early. Within a few hours of exposure to less than 0.5 ppm ozone—a concentration readily achieved at peak traffic hours in some urban areas—considerable reaction becomes apparent. Within 4 hours of exposure to about 0.9 ppm ozone ($O^3$)—a level occasionally reached in heavy smog—cellular changes are obvious. Mixtures of as little as 0.25 ppm $O^3$ and 2.5 ppm $NO^2$ caused definitive injury within 24 hours to the lining cells of the lung's small airways. A mixture of the two compounds at the high ambient level of 0.9 ppm $O^3$ induced an advanced, lethal degree of emphysema within 2 months upon continuous exposure.

Because the lung (a model of a normal rat lung is depicted) is the primary site of chemical toxicity, NIEHS researchers strive to determine how it handles exposure to multiple agents.

In pathologic studies, Dr. Donald Dungworth at the University of California at Davis found that the focal points of damage at all levels of exposure in the rat are the small airways. Exposure to as little as 0.2 ppm ozone for 7 days causes mild but unequivocal damage. Dead cells and debris accumulate at these points, digestive enzymes are released, and changes in lining cells are seen easily by light or electron microscopy. Starting about 2 hours after beginning exposure to either 15 ppm $NO^2$ or 0.5 ppm $O^3$ the sequence of cellular effects involves a sloughing of ciliated cells and of the thin, flat Type I cells from the alveolar walls. This accounts for the accumulation of cellular debris. However, upon short-term exposure at this level or long-term exposure at considerably lower levels, replacement of epithelial cells is accelerated, reaching a maximum during the 2nd and 3rd days and returning to the pre-exposure rate within a week. The new cells that replace the injured ones are resistant to the previously toxic concentrations of the gases. Studies are in

progress to determine why these cells are resistant. Research on other gases such as sulfur dioxide indicate induction of resistance may not be restricted to ozone or nitrogen dioxide but may be a general response to damage.

Insight into molecular bases of cellular injury has been provided by Dr. Daniel Menzel at Duke University, Dr. Bernard Goldstein at New York University Medical Center, and Dr. Orville Privette at the University of Wisconsin, as well as by Drs. Freeman, Stephens, and Dungworth. Experiments by Drs. Menzel and Privette show that ozone exposure results in changes in polyunsaturated fatty acids and support the hypothesis that ozone and nitrogen dioxide induce membrane and cellular degeneration by means of lipid peroxidation.

Dr. Goldstein also showed that free radicals, peroxides, and carbonyl compounds are formed from unsaturated lipids. His work suggests that the carbonyl compounds which begin to circulate in the blood stream shortly after ozone exposure may serve as a basis for early detection of ozone damage by simple chemical techniques for measuring elevated carbonyl content of blood samples.

Other potentially important biochemical changes (which are in accord with progression of tissue degeneration to the gross pathological endpoint seen in chronic obstructive lung disease and emphysema) occur as a consequence of lipid peroxidation and membrane changes. Prostaglandins, or biologically active substances resembling hormones, are released in a manner similar to their release in general tissue damage (Dr. Privette). Age-related pigments called lipofuscins are induced (Dr. Saari Csallany, University of Illinois). Pulmonary tissue oxygen consumption is increased with an attendant increase of about 30 percent in the number of specific enzymes involved in regulation of cellular metabolism and in digestive enzymes capable of breaking down lung tissue (Dr. Dungworth). Cytoplasmic thiols are oxidized and certain soluble proteins are polymerized (Dr. Menzel).

While all of these studies clearly establish the sequence of events in ozone and nitrogen dioxide induced disease, the research of other NIEHS grantees suggests that much if not all of the damage induced by these two pollutants at low levels can be prevented and perhaps reversed in the early stages.

Dr. Goldstein has demonstrated that various compounds, including allylisopropylacetamide (AIA) and para-aminobenzoic acid protect against ozone toxicity. Lipid antioxidants, particularly vitamins A and E, also have been shown to afford such protection, suggesting that these and other natural antioxidants may be useful for preventive or therapeutic purposes. Certain minerals, particularly zinc, also may be useful as Dr. Milos Chvapil at the University of Arizona has shown that zinc ions and certain zinc chelate complexes markedly stabilize lysosomes which are sources of collagenase and other digestive enzymes. Dr. Dungworth has discovered that peroxides formed upon lipid peroxidation are detoxified or made less harmful by means of the glutathione peroxidase system. Some enzymes in this system were shown to be induced by as little as a 7-day exposure to 0.2 ppm ozone. Thus, the pulmonary response is very sensitive and may be important as an adaptive or inducible defense mechanism.

It seems appropriate now to proceed with clinical evaluations of AIA, para-aminobenzoic acid, vitamins A and E, zinc, and combinations of these in test animals and eventually human populations at risk of toxic exposures to nitrogen dioxide and ozone.



### Potential Teratogens

NIEHS studies with DES (diethylstilbestrol), a synthetic hormone, have emphasized the unique sensitivity of the embryonic period to chemical insults that may result in infertility and perhaps cancer later in life.

Although many compounds are continously introduced into the environment, few have been examined for their potentially toxic effects on reproduction and development. Virtually nothing is known about the effects of exposures to common drugs and chemicals before birth on the development of the offspring after birth.

Dr. John A. McLachlan and his co-workers have been using mice to study the effects of environmental agents on reproductive tract function. In their work with DES, a chemical used as a livestock food additive and postcoital contraceptive, the scientists demonstrated that exposure to DES before birth adversely affects the reproductive capacity of the female offspring. This effect is dose-related and is due in part to the relative inability of the female offspring to ovulate. During the prenatal period oocytes (reproductive cells) undergo division in man and in laboratory rodents, and at that time they are especially susceptible to chemical intervention.

The researchers also found that the male

offspring are sterile following prenatal exposure to certain doses of DES. Tests suggest that DES affects the same embryonic tissues in both male and female fetuses and results in postnatal defects in both sexes. The reproductive tracts in the prenatally drug-exposed offspring have lesions which include changes in cell types and/or tumors.

In light of these results in rodents the incidence of genital tract abnormalities in young men whose mothers had been treated with DES



Mouse offspring exposed before birth to DES, a synthetic hormone, have been found to have lesions, including tumors, in their reproductive tracts. This finding emphasizes the unique sensitivity of the embryonic period to chemical insults.

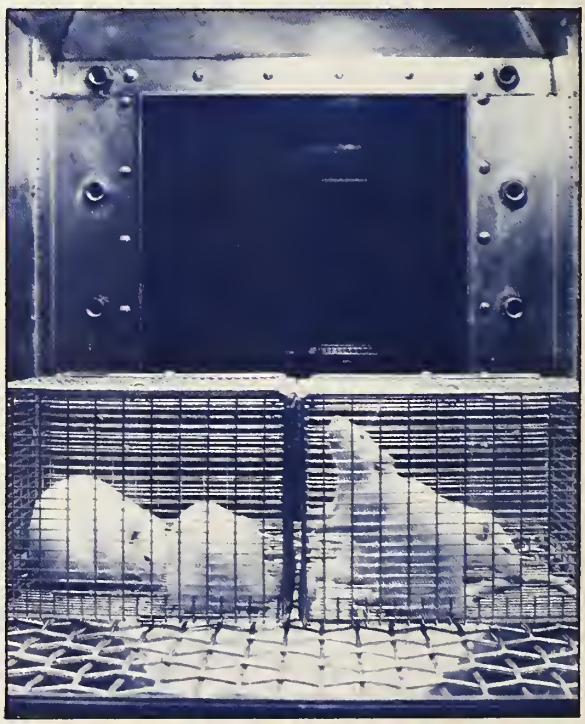
during pregnancy may be of clinical importance. Up to now effects of prenatal exposure to DES in the male offspring of treated mothers have not been clinically described, in contrast to findings reported in females. These results may also be pertinent to the development of an animal model to study the reproductive tract lesions reported in young women whose mothers had been given DES during pregnancy to prevent abortion. This would permit more rapid testing of its teratogenic activity in the search for a way to predict toxic effects.

In other work Dr. F. D. Andrew determined that the progestational steroid medroxyprogesterone acetate (MPA, Provera®), a suspected teratogen in humans, results in offspring with cleft palate when administered to pregnant rabbits. In contrast teratogenicity was not noted in the offspring of rats or mice given MPA at even higher doses. Palate malformations rarely occur spontaneously and have seldom been induced experimentally except by glucocorticoids, a different class of steroid hormones. In view of the obvious species difference and the unusual occurrence of palatal malformations in the rabbit, the teratogenic mechanisms of MPA are being explored further in rabbits. Knowledge gained from these studies can improve our ability to predict fetal toxicity and/or teratogenicity.

## Airborne Hazards

The respiratory tract is the route of entry for airborne environmental agents and represents the first line of defense. Because the body confronts not one but multiple agents, it must be determined if there are additive or synergistic effects from the combined exposure and if this becomes a factor in producing toxic effects. NIEHS' Dr. Robert T. Drew has developed a program to determine the effects of exposure to inhaled materials, singly and in combination.

Sulfuric acid mist and ozone were the first combination of compounds to be investigated under a contract supervised by Dr. Finis Cavender of Becton, Dickinson and Company Research Center, Research Triangle Park, N.C. They were selected because of their presence in ambient urban atmospheres and because sulfuric acid mist is a major by-product of the catalytic converters now being used widely as antipollutant devices on automobiles.



Acute and chronic studies are being carried out with NIEHS support in an attempt to determine the effects resulting from exposure to inhaled materials, such as the pollutants commonly found in our atmosphere.

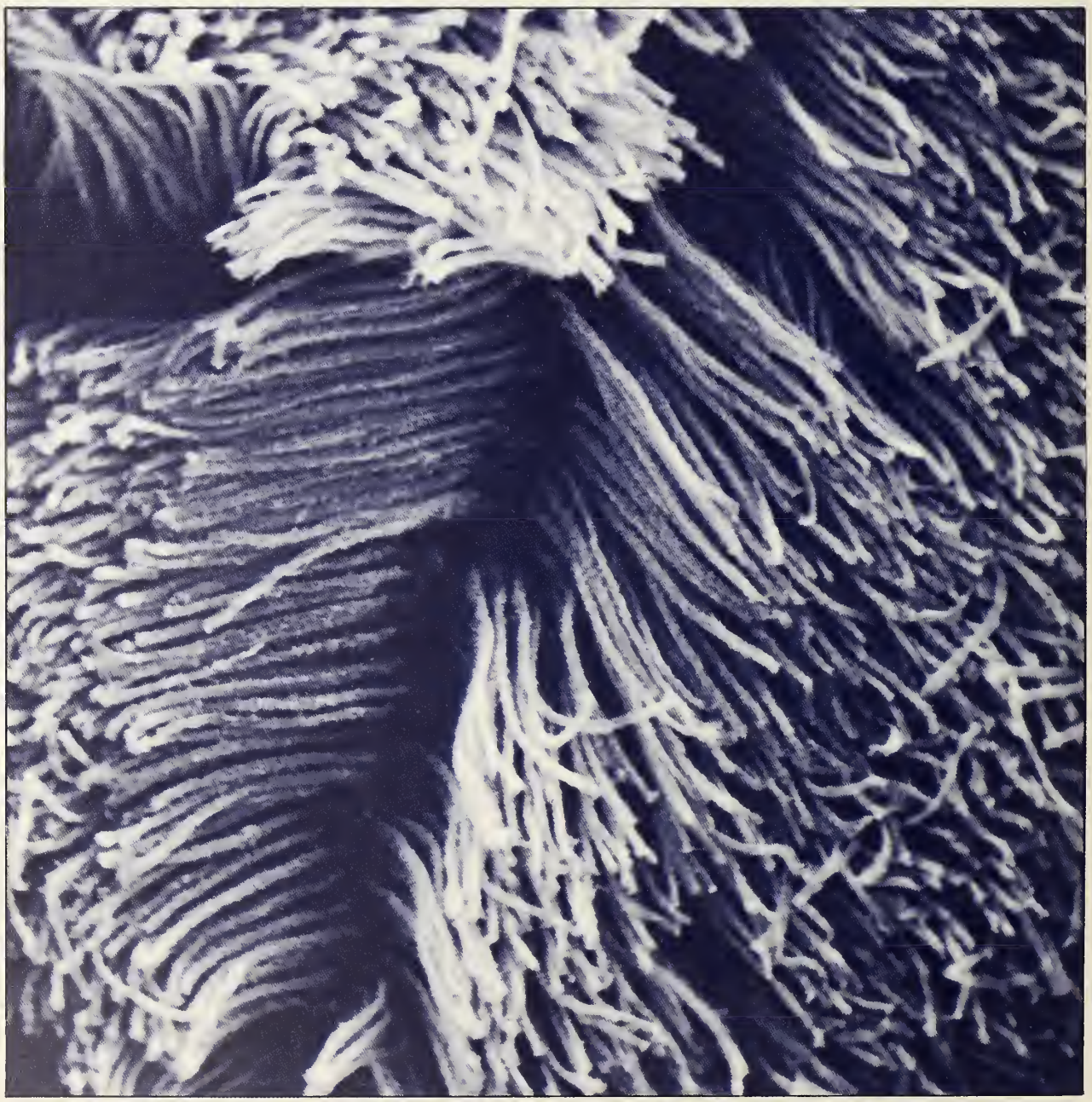
A series of preliminary subacute studies have been completed at various concentrations of sulfuric acid mist and ozone to determine any synergistic effects. These studies suggest that while effects can be seen from both compounds there is little or no evidence of a greater-than-additive effect. However, a remarkable species difference was confirmed in guinea pigs and rats with respect to susceptibility to sulfuric acid mist. Guinea pigs are sensitive to the pollutant while rats are resistant, and studies are in progress to determine the reasons for this difference. It will be important to determine whether man responds more nearly like the rat or the guinea pig to sulfuric acid mist.

Studies are underway to investigate the effects of particle size, acid concentration per particle (molarity), and numbers of particles on toxicity of sulfuric acid mist. Preliminary results suggest that sulfuric acid mist particles of about 1 micrometer in size generated from a 5 molar solution produce maximum effects. Chronic studies are in progress with rats and guinea pigs exposed to 0.5 parts per million ozone and 10 milligrams per cubic meter sulfuric acid mist to determine their potential synergistic effects.

Animals exposed to these compounds will be compared to unexposed animals with respect to many kinds of biological indicators, including growth rate, mortality, structural changes, changes in respiratory physiological indices, and changes in rates of pulmonary metabolism. Results of these investigations should help determine the threat to man's health from exposure to combinations of environmental pollutants in the air we breathe.

Photoreceptor cells (rods and cones) of the monkey retina — a scanning electron microscopic view.





## New Technique to Visualize Blood Flow in Ocular Vessels

Development of a new method for photographing simultaneously blood flow in the vessels of the retina and underlying choroid may be an important new tool in the diagnosis of several eye disorders. At present, diagnosis is complicated by the apparent similarity of many such diseases and by the lack of differential tests to distinguish among them.

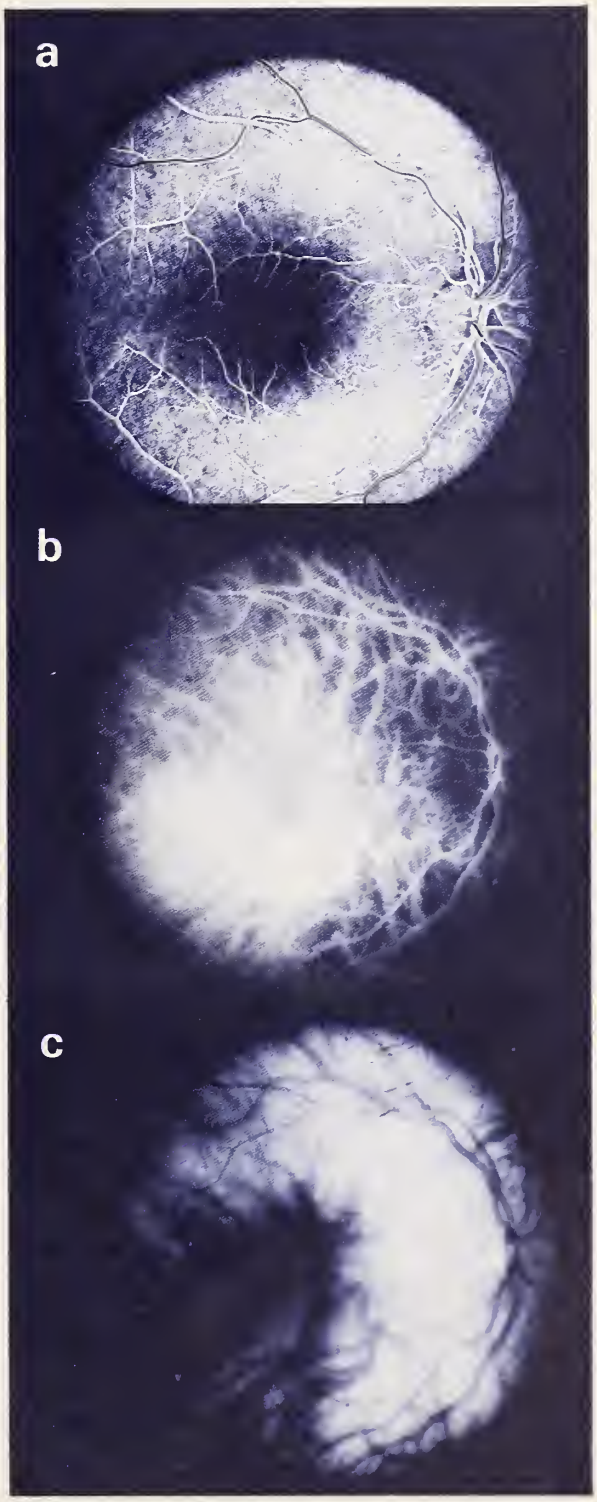
More than one-third of all blindness in this country is caused by disorders of the light-sensitive retina and the closely-related choroid. Both are subject to the same systemic influences which affect other such tissues in the body as well as to those unique to the eye. Research related to retinal and choroidal disorders has been slowed by lack of knowledge about the structure and function of the eye's two major and interrelated blood circulation systems.

Of the many diseases which can strike the retina and choroid, three in particular—diabetic retinopathy, senile macular degeneration, and sickle cell retinopathy—share the characteristic of impaired or altered blood circulation. In addition, the latter two appear to have an affinity for particular regions of the retina—the macula (the area of central, sharpest vision) and the midperipheral area.

Why these regions of the retina are susceptible to diseases related to impaired circulation and what metabolic activities are involved may be explained by research supported by the National Eye Institute at Johns Hopkins University. In collaboration with R.W. Flower of the Johns Hopkins Applied Physics Laboratory, Dr. Arnall Patz and associates at the hospital's Wilmer Opthalmological Institute have developed a technique to study the interrelationship of the blood circulations of the retina and choroid. Their technique makes it possible for the first time to photograph simultaneously the individual passage of each of two dyes through these separate systems after a mixture of the dyes has been injected into an arm vein.

In a technique called fluorescent angiography, the flow of fluorescein dye through the retinal blood vessels can be photographed; however, this dye does not provide good visualization of choroidal blood flow because of the tissue's thickness, the presence of pigment, and other factors.

But, the Johns Hopkins investigators have found that another dye—indocyanine green—which fluoresces at a different wavelength than fluo-




Fluorescein angiography visualizes blood circulation in retinal vessels. Simultaneous angiograms of a human eye show (a) fluorescein dye as white lines against the background of the choroid, (b) indocyanine green dye (ICG) illuminating the network of choroidal vessels and, (c) absorption of light energy by ICG as black lines in larger vessels.

rescein provides good visualization of the choroid's dense circulatory system, which carries oxygen and nutrients to part of the retinal tissue.

Three motorized cameras attached to a modified fundus camera permit separate but simultaneous photography of the two circulatory systems. Filters and optical beam splitters separate and direct the different wavelengths emitted by the dyes to the separate cameras. Two cameras provide simultaneous information about the blood flow in retinal and choroidal vessels while the third camera records absorption of light energy by indocyanine green.



Simultaneous angiography is now being used on a limited basis at the Johns Hopkins School of Medicine with some patients who have diabetic retinopathy and sickle cell retinopathy, disorders of the ocular blood vessels related to systemic disease, which can result in severe visual impairment and blindness. It is hoped that the technique will provide new information about the interaction of the two circulatory systems in these disorders and how changes in blood flow might lead to visual impairment. Such knowledge may help in the development of improved methods of diagnosing, treating, and perhaps preventing diabetic and sickle cell retinopathy.

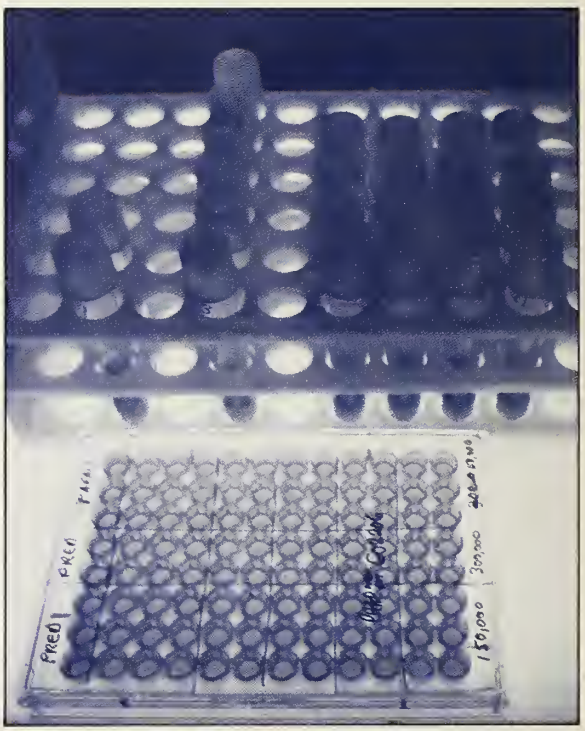
In addition, simultaneous angiography may be a valuable tool in investigations aimed at defining the primary site of degeneration of the macular region of the retina, a major cause of loss of central vision and blindness.

The Johns Hopkins scientists are also using simultaneous angiography to help study the role of oxygen in normal retinal function and in certain diseases. This third area of research is aimed at determining the survival time of various retinal layers following deprivation of normal oxygen and glucose levels. The study is clinically significant because it explores the possibility of supplying oxygen to the retina following retinal arterial occlusion by temporarily increasing oxygen delivery capacity of the choroid until retinal circulation is restored.

### Evidence of a Metabolic Defect in Glaucoma

Studies linking a systemic response to corticosteroids with the occurrence of primary open-angle glaucoma are providing an important new approach to research on this major cause of visual disability and blindness. Such investigations may lead to a clearer understanding of the cellular basis for this disorder and perhaps even to better means of treatment.

All forms of glaucoma are characterized by elevated pressure within the eye, damage to the optic nerve, and subsequent defects in the field of vision. In the most common form, primary open-angle glaucoma, the juncture where the cornea meets the iris, through which aqueous fluid flows, appears to be normal. Yet, aqueous flow out of the eye is blocked, causing a buildup in intraocular pressure which damages the optic nerve. The site of outflow resistance is believed to be within the tiny drainage channels which lie beyond the angle.



Laboratory tests link a systemic response to corticosteroids with primary open-angle glaucoma.

Most cases of primary open-angle glaucoma can be controlled through regular administration of medication usually in the form of eyedrops, which either decrease the amount of aqueous fluid produced, facilitate its drainage from the eye, or both. But, because visual loss from this form of glaucoma is usually so gradual, it may remain unnoticed by the patient until considerable damage has occurred. And, because in some cases elevated pressure cannot ultimately be controlled either by medication or last-resort surgery, glaucoma remains a leading cause of blindness.

Primary open-angle glaucoma may be associated with a genetically-determined sensitivity of

the eye to corticosteroids. Topical administration of steroid drugs causes a marked elevation in intraocular pressure in 81 percent of individuals with primary open-angle glaucoma and in 6 percent of individuals without the disease. Prolonged administration of these drugs to some individuals without glaucoma, such as in the treatment of chronic inflammatory diseases, can produce a condition which mimics primary open-angle glaucoma, including irreversible visual loss.

In 1972, Drs. John F. Bigger, Paul F. Palmberg, and Bernard Becker of Washington University School of Medicine in St. Louis demonstrated that lymphocytes, one of the white blood cell types, from glaucoma patients as well as from individuals who have a marked ocular steroid response are more "sensitive" to steroids than those from people without glaucoma or who do not have the ocular response.

The substance phytohemagglutinin-P (PHAP) can transform normal, inactive lymphocytes to a metabolically active state in the test tube. Certain steroid hormones are known to inhibit this transformation. The Washington University investigators reported that only half the concentration of the steroid prednisolone was required to inhibit by 50 percent the transformation of lymphocytes from glaucoma patients and high ocular steroid responders than was needed to inhibit the transformation of those from other patients. This was the first demonstration of a differential sensitivity to steroids outside the eye and in isolated cells in glaucoma patients, providing evidence of a biochemical basis for this disease.

Last year, the St. Louis investigators reported a high correlation between varying degrees of corticosteroid sensitivity in lymphocytes and the ocular response to topical steroids in a group of 100 patients, providing additional evidence that differential corticosteroid sensitivity is genetically determined and affects many tissues in the body. Lymphocytes from individuals who do not develop a rise in intraocular pressure after topical steroid application appear to be the least sensitive, whereas those from people with the most marked ocular pressure response (glaucoma patients and others) are the most sensitive.

The lymphocyte transformation inhibition assay is not able to distinguish an individual with diagnosed glaucoma from one who merely has an ocular response to topical steroids. Nor does the test discriminate high ocular responders who usually have normal intraocular pressure from those who have high pressure prior to steroid testing.

Nevertheless, the lymphocyte test, performed in the test tube without need for administering steroid drugs over a number of weeks to provoke increased pressure in a functioning human eye, offers obvious advantages for studying the cellular basis of elevated intraocular pressure.

In addition, the test may be useful for distinguishing among different types of glaucoma. Most recently it has been used by the Washington University scientists and their associates to find out whether pigmentary glaucoma, in which there is marked pigment dispersion in the front portion of the eye, is a separate disorder from primary open-angle glaucoma. Twenty patients with pigmentary glaucoma did not show the markedly increased cellular sensitivity to corticosteroids associated with primary open-angle glaucoma. These results indicate that pigmentary glaucoma is a distinct clinical entity with a different cause from that of primary open-angle glaucoma.

If a specific metabolic or enzymatic defect related to glaucoma can be demonstrated, it will also furnish a rational basis for developing new drugs which may be effective in treatment or even prevention of this disease.

## Role of Lens Protein Aggregation in Cataract Formation

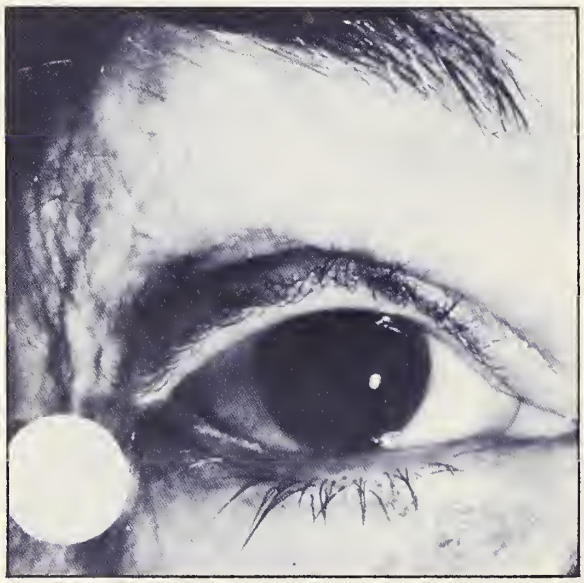
Surgery is now the only treatment for cataract. However, recent studies of how cataracts form are hastening progress toward development of a nonsurgical means of treating or preventing the most common type which occurs predominantly among the elderly and is a leading cause of blindness.

Understanding how lens transparency is normally maintained and elucidating the process leading to opacification of the lens are high priority goals of cataract research. Recent

National Eye Institute-supported studies by Drs. Abraham Spector and Joseph Stauffer at Columbia University and Drs. George Benedek, Judith Jedziniak, and Toyoichi Tanaka at Massachusetts Institute of Technology indicate that size and configuration of lens proteins are important factors in determining lens transparency.

Four years ago it was suggested that clumping or aggregation of protein molecules, which occurs in senile cataract, is responsible for loss of lens transparency because the aggregates scatter light. Soon after, investigators uncovered actual evidence that protein aggregates might exist in aging normal as well as cataractous lenses. However, because their studies involved materials extracted from lenses and separated biochemically, the researchers considered it important to determine if protein aggregates also exist in the intact lens.





A cataract, shown outside the eye, blocks passage of light to the retina, and may result from protein clumping associated with aging of the normally clear lens.

Resolution of this question came only within the past year. By measuring over a period of time the spectrum of laser light scattered by intact lenses, the investigators were able to confirm the presence of protein aggregates and determine the sizes of different particles. They found that aggregates in the cataractous lens are over 50 times larger than normal lens protein, a measurement that corresponded to the protein size determined earlier in the lens extracts.

The demonstration that the size of proteins can be determined optically has provided a means for studying cataract formation in intact normal as well as cataractous lenses.

Scientists are now working to determine the cause of protein aggregation in the lens. In particular, they have investigated alpha-crystallins, one class of lens proteins thought to play a role in the development of senile lens opacities. Studies of animal alpha-crystallin had already shown that this large class of proteins is composed of macromolecules of various sizes. Other investigations have demonstrated that at least one type of human senile cataract—the nuclear sclerotic form—is associated with a reduction in the content of low molecular weight proteins concomitant with an increase in insoluble protein. This finding has led to the concept that cataract formation is associated with conversion of low molecular weight protein to high molecular weight aggregates, the heaviest of which correspond to the insoluble or albuminoid portion of the proteins. Further investigations have made it possible to calculate the molecular weight of protein necessary to cause lens opacity and have verified that conversion of alpha-crystallin to high molecular weight aggregates does occur.

Investigators studying the development of high molecular weight proteins in human lenses found that this phenomenon occurs in only one part of the lens and is directly associated with the aging process. Examination of normal human lenses from people 3 months to 89 years old indicated a gradual increase in the percent of high molecular weight proteins in the soluble portion of the lens, beginning in the early teens.

Investigators therefore concluded last year that the high molecular weight aggregation is almost entirely exclusive to the nuclear region of the lens and, furthermore, that the increase in scattering of light caused by the aggregates parallels an increase in high molecular weight proteins in this region.

Studies also conducted last year indicate that calcium may play a role in protein aggregation. Earlier work had shown that there is a marked increase in calcium ion concentration concomitant with development of lens opacities. There is further evidence that transformation of low molecular weight protein to high molecular weight aggregates may be induced by an interaction with calcium.

If it is proven that calcium-induced aggregates do contribute to cataract formation, it may be possible to identify means of controlling the calcium and inhibiting the aggregation process.

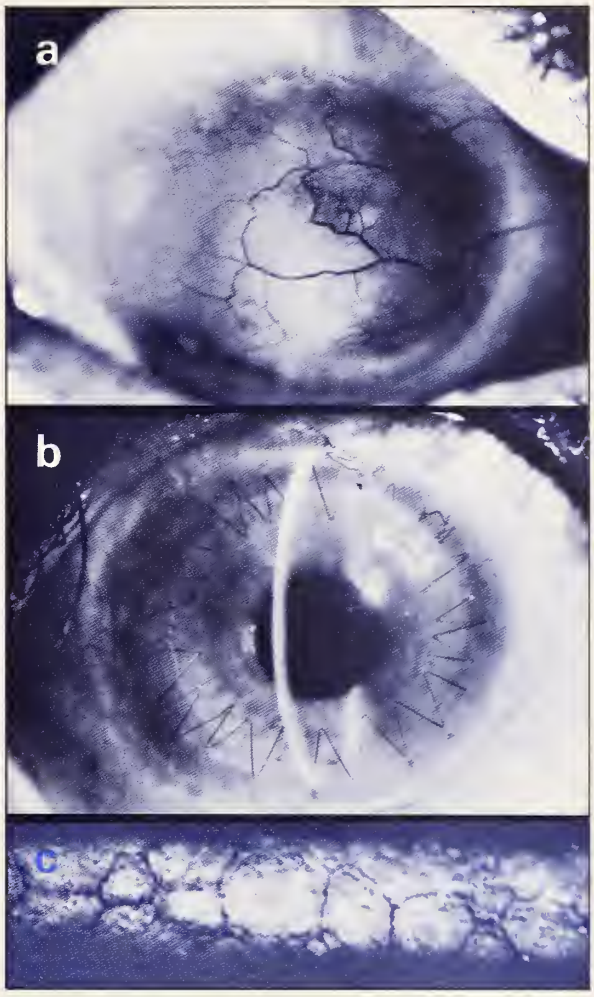
## Tissue Culture Extends Life of Donor Corneas for Transplantation

Transplantation of the cornea may soon become more successful and its benefits extended to more people because of a new method for prolonging the life of fresh donor tissue.

Vision that is lost when the normally transparent tissue at the front of the eye becomes scarred or opaque can often be improved or restored by replacing a damaged cornea with a clear healthy one. However, the usefulness of corneal transplantation has been limited because fresh tissue can be viably maintained by refrigeration for only a short time—not more than 48 hours. This makes it difficult to obtain donor corneas to be obtained when they are needed or, conversely, to schedule surgery on short notice when tissue suddenly becomes available. Furthermore, even within the 48-hour period, vital corneal endothelial cells may begin to deteriorate. These cells function as a biological pump, removing water from the cornea, an action essential for maintaining its transparency. Loss of these cells, not always apparent prior to transplantation, causes the grafted tissue to swell and become opaque. At least half of all unsuccessful grafts caused by tissue failure may be due to loss of these cells.

Vision research scientists have therefore sought means of both extending the preservation period and maintaining the viability of the endothelial cells. Both goals may be achieved by a recent advance in corneal research at the University of Florida. Drs. Bernard E. McCarey and Herbert E. Kaufman, working with National Eye Institute support, have developed a simple inexpensive technique that preserves a fresh donor cornea for as long as a week in a solution they call M-K media. In developing the media, the Florida researchers sought to avoid the complexity and expense of other methods which have been used in attempts to extend the period of corneal preservation. The M-K media consists of a readily available standard tissue culture solution (TC-199) to which dextran and the antibiotics streptomycin and penicillin are added. The media satisfies the ionic and nutritive needs of the cornea and counteracts the natural tendency of the tissue to swell; the antibiotics prevent or retard possible infection.

Preparing a cornea for eye bank storage with the new media is relatively simple, and equally important, minimizes handling of the tissue and the risk of error. Enucleated donor eyes are




(a) A severely burned and damaged cornea before penetrating keratoplasty. (b) A transplanted cornea which had been preserved several days in M-K medium is clear 24 hours after surgery. (The white arc is a reflection from a slit lamp biomicroscope.) (c) The specular microscope reveals an intact and apparently normal endothelium as early as 24 hours after keratoplasty.

bathed and immersed in an antibiotic solution before the cornea is removed. The tissue is then placed in a large volume of fresh M-K media and subsequently immersed in a vial of the solution for storage and shipping. When needed, the tissue can be used directly from the vial without further preparation.

The McCarey-Kaufman technique, with its simplicity and economy, also overcomes a drawback to cryopreservation, a method to freeze-dry donor tissues. Cryopreservation, developed 10 years ago with NIH support, promised to extend the potential of corneal transplantation by permitting indefinite storage. Although successful, cryopreservation involves

complex and expensive equipment which requires highly skilled technicians and strict adherence to procedure. Difficulty in shipping the frozen tissues has further limited its use to a few large medical centers.

In addition to prolonging the life of donor tissue, the new media also has a distinct advantage over refrigeration in that only the cornea, rather than the entire eye, is preserved. Whole-eye preservation may cause contamination of the cornea by the catabolic processes which continue in the enucleated eye; and in particular, may contribute to the steady deterioration of the cornea's vital endothelial cells.



To reduce this cause of tissue failure, the University of Florida team concentrated on developing a method that would extend not only the corneal storage period but also the survival time of the endothelial cell layer. A recent study by Johns Hopkins University has found that the M-K media accomplishes this aim so successfully that now, all donor tissues at that institution, including those to be transplanted immediately, are prepared utilizing the M-K method.

Although the McCarey-Kaufman technique is only about a year old, eye banks throughout the United States are using the procedure for corneal preservation. Moreover, the researchers believe that their technique may eventually provide a possible means of storage for other body organs and tissues to be used in transplantations.

### Influence of Early Experience on Maturation of Visual Nervous System

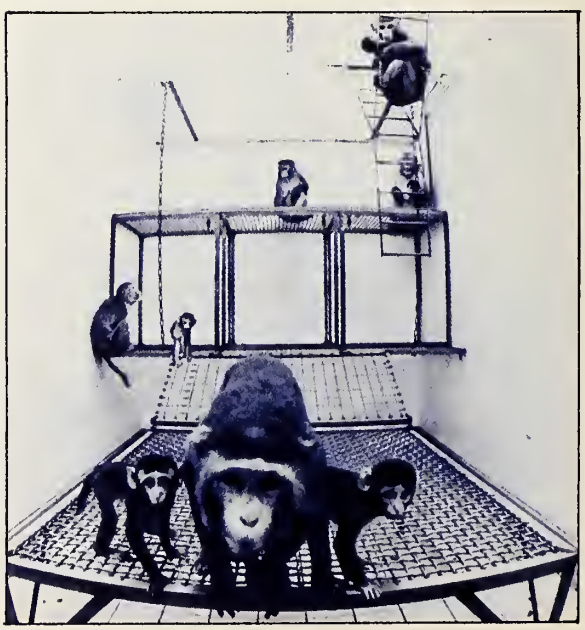
New evidence substantiates the theory that environmental experience early in life is important for the full maturation of the visual nervous system. Recent studies by Drs. David H. Hubel and Torsten N. Wiesel of Harvard Medical School, have shown that while innate mechanisms are largely responsible for the development of the visual system, deprivation early in life can adversely affect its proper functioning.

Drs. Hubel and Wiesel's work, which is supported by the National Eye Institute, is broadly directed toward delineating those environmental and genetic factors important to the normal development of the mammalian visual system. In particular, they are concentrating on better understanding the functional architecture of the visual cortex in normal and visually-deprived animals.

Several years ago, Drs. Hubel and Wiesel began studying the anatomy and physiology of the mammalian visual cortex. Their work, first in cats and later in monkeys, provided the first architectural description of the striate cortex, the visual area of the cerebral cortex, that correlated with the functional role played by this section of the brain.

Early investigations by this team made use of electrode implants to study the brain's response to visual stimulation. Later, they began using radioisotopes and most recently progressed to a sophisticated new cellular staining procedure developed by a member of their staff. This refined method enabled the researchers to study more precisely the architectural development of the monkey striate cortex, which is very similar to that of humans.

Their studies with infant monkeys revealed that the visual cortex contains two separate but overlapping functional systems. These are the so-called "orientation" columns, which contains cells specific to stimuli of a particular position, shape, size, or direction of movement, and the "ocular dominance" columns whose cells are specific to either one eye or the other. Both, they found, are highly ordered and arranged in parallel sheets within the cortex. They applied this knowledge to the study of young macaque monkeys with little or no visual experience and found their orientation systems to be intact, a result which conclusively demonstrated that the ordered column system, itself, is innately determined and not the result of visual experience early in life.



Even though Drs. Hubel and Wiesel found the overall orientation system as well as most of these cells in the monkey striate cortex to be normal, approximately 10 to 15 percent of the cells they tested showed some abnormalities in their pattern of response to visual stimuli. This, they presumed, was due to the visual deprivation. In addition, the monkeys lacked some cells necessary for binocular vision. Because the normal, two-day-old monkey is known to have the necessary assortment of binocular cells, the investigators determined that binocular deprivation early in life leads to distinct loss of those brain cells influenced by both eyes.

Other studies have shown that deprivation of sight in only one eye shortly after birth causes the columns of cortical cells specific to that eye to shrink in width. At the same time, this leads to an increase in the width of columns receiving input from the functioning eye.

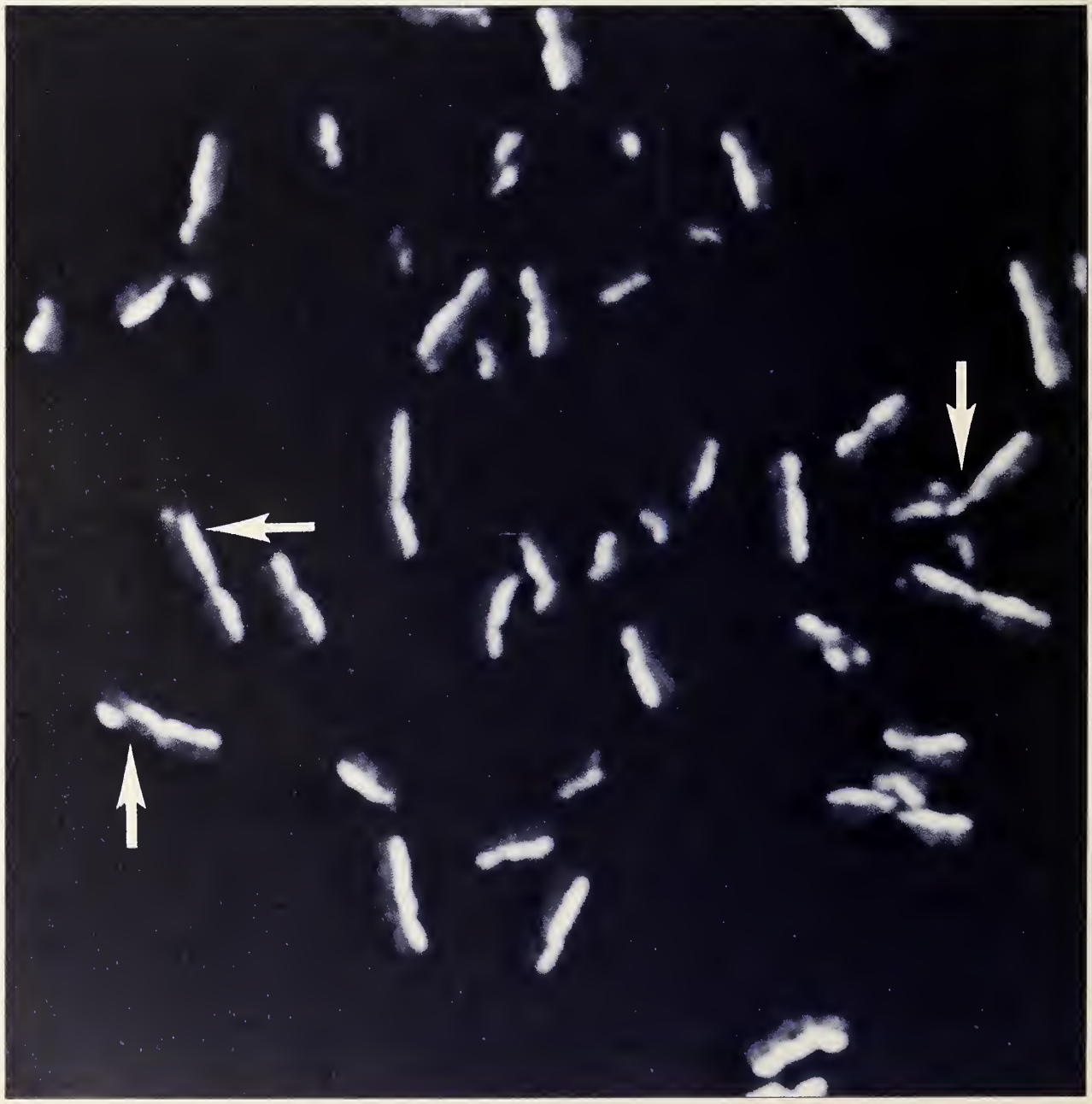
These findings strongly indicate that the visual system is flexible and plastic and, although most of the structure and function of the cortex is genetically determined and present at birth, visual experience is important in shaping the brain's response to stimulation.

Identification of the independent but overlapping orientation and dominance column systems has led Drs. Hubel and Wiesel to suggest that there may be other aggregations of specific cells in the striate cortex, such as a column system for color coding. They believe that it should be possible to analyze the aggregation of cells in the cortex and identify other independent and overlapping systems.

Implications of this research range from improved understanding of the normal visual process to direct clinical applications. For example, continued efforts may lead to a more complete explanation of those factors involved in childhood visual disorders such as amblyopia—loss of visual function associated with disuse of one eye—and strabismus—misalignment of the eyes—as well as other important causes of visual disability and blindness.

Visualization of sister chromatid exchanges occurring in DNA repair provides a new means to assess hazards of environmental agents on human genetic material. Arrows point to several exchanges of sister chromoid DNA in newly replicated chromosomes from a normal human lymphocyte.





## Cell Recognition and Adhesion

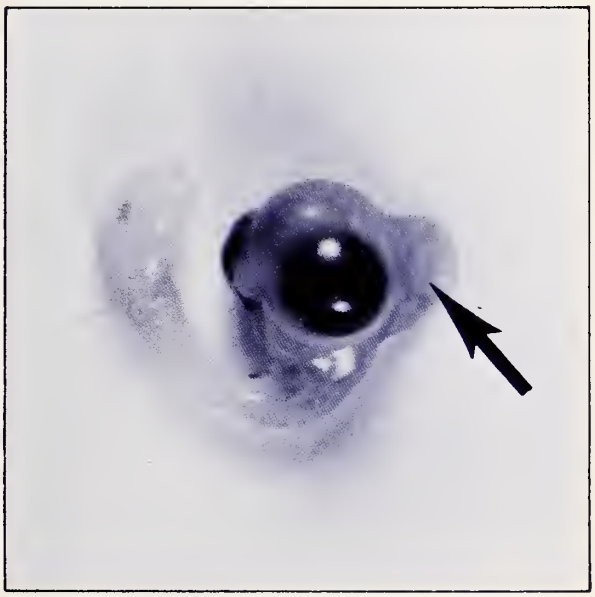
How do cells of a tissue, such as the retina of the eye, recognize each other? What holds them together in a characteristic recognizable and functional structure; why do cancer cells lose this and other normal properties? To answer these questions, National Institute of General Medical Sciences grantee, Dr. Luis Glaser, and his associates at Washington University School of Medicine in St. Louis, have performed a series of experiments on membranes from embryonic chick retina cells.

Previous work had shown that a sample of retina can be separated into a suspension of individual cells by a gentle chemical treatment. If the cells are then allowed to settle in fresh medium, they aggregate and eventually reproduce many features of the original tissue. Furthermore, if two different tissues, such as brain and retina, are dispersed into suspensions of single cells, and then mixed and allowed to reaggregate, a slightly different process occurs. First, cells of both types clump together in large groups. Soon, however, they begin to sort out into two different regions, and finally to form two entirely separate aggregates: one of brain cells, and the other of retina cells. Scientists believe that this ability of like cells to recognize and specifically attach to each other is due to molecules found on their surfaces called cell-surface recognition sites.

As a first step in determining the chemical nature of these sites, Dr. Glaser and his co-workers isolated cell membranes from chick embryo brain cells and from retina cells. In order to demonstrate that the isolated membranes had retained the cell-surface recognition sites and that these sites retained their specificity (ability to recognize and attach to the like cell type), they took *membranes* isolated from 8-day chick retina cells and mixed them with a suspension of *whole* single cells from the retina. The membranes became bound to the retina cells, suggesting that the isolated retina membranes had retained their cell-surface recognition sites. The next step was to determine whether this binding of retina cell membranes to disaggregated retina cells prevented formation of retinal aggregates. This would suggest that the membranes were attaching to the cell-surface recognition sites. Dr. Glaser's group found that mixing the retina membrane preparation with the *whole* disaggregated retina cells almost totally inhibited the clumping or aggregation of the retina cells.

The next questions to be answered concerned specificity: were the cell-surface recognition sites on the isolated membranes specific, that is, capable of recognizing only the correct cell type? Dr. Glaser performed parallel experiments in which he mixed the isolated retina cell membranes with suspensions of whole retina cells in one vessel and with suspensions of whole brain cells in another. In the first test, the retina membranes bound to the retina cells, and in the second test, retina membranes did not bind to the brain cells.





Studies of a single cell isolated from chick embryo brain tissue give evidence that rapid changes in structure of molecules found in the cell surface membranes are a major key to the formation of different tissues and organs during embryonic growth and development. In this photo of a seven day embryo, the arrow points to the portion of the brain from which single cells are prepared for aggregation assays.

Scientists had previously proposed that during the formation and growth of an embryo, cell-surface recognition sites such as those demonstrated by Dr. Glaser might be responsible for the formation of tissues in the embryo as well as for the changes to more specialized and complex tissues as growth of the embryo progresses. This could be accomplished if the cell-surface recognition sites change during development, thus allowing cell associations to change. Dr. Glaser and his co-workers tested this possibility by isolating membranes from retinas of 8-day old chicks, and testing their ability to bind to and inhibit aggregation of intact retina cells from 7-day, 8-day, and 9-day old chicks. Their results show that the membranes from retinas of the 8-day chicks bound strongly to the whole 8-day retina cells and prevented them

from aggregating. However, binding of membranes from 8-day retina cells to either 7-day or 9-day retina cells either did not occur or was very weak or infrequent, since aggregation of these cells was only slightly inhibited by the presence of the 8-day retina cell membranes.

This provides strong supporting evidence that the cell-surface recognition sites on these cells change rapidly during development. Dr. Glaser is now attempting to isolate cell-surface recognition site material from the membranes in order to determine its chemical composition and structure. It is hoped that the study of these molecules in normal tissues will elucidate the changes in cancerous tissues.



### Basic Approaches to Cancer Diagnosis

To aid in the early detection of cancers, scientists and clinicians are constantly seeking new tests for the presence of cancerous tissue. The initial finding, in 1965, that a material called carcinoembryonic antigen (CEA) could be detected in the serum of patients with digestive system malignancies but not in the serum of normal individuals or patients with other cancers, led investigators to hope that CEA assays might provide such a diagnostic test.

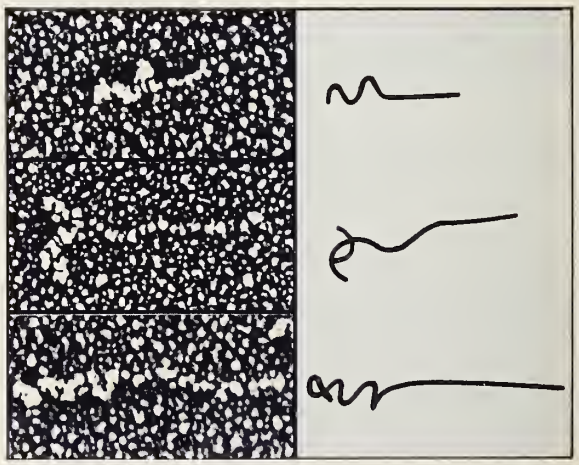
Embryonic tissues contain a number of substances that are absent, or present in very low concentrations, in adult tissues. Some malignant tumors in adult animals contain high concentrations of these molecules which are usually found only in fetal tissues. Carcinoembryonic antigen, a glycoprotein, is one example of such a substance. This antigen can be detected at the surface of tumor cells, although it does not appear to be an integral part of the membrane and readily passes into the circulation where it can be detected by a test using radioimmunoassay techniques.

Although the initial work on CEA suggested its use in the detection and diagnosis of cancer, further work has shown that CEA is present at low levels in normal adult tissues, normal blood, and disease states other than cancer. Thus its most promising application in the immediate future is in the management of a patient with a known malignancy both during and following therapy, by the use of a series of CEA assays. This use demands assays for CEA that are highly specific and quantitative.

In order to produce more specific antisera to be used in the radio-immunoassay, extremely pure preparations of CEA must be made. This has been difficult to do by conventional methods.

Dr. Henry S. Slayter, at the Sidney Farber Cancer Center in Boston, with support from NIGMS and the Division of Research Resources, has been working with a number of collaborators to identify and distinguish carcinoembryonic antigen with the aid of electron microscopy. They theorized that, if a distinctive molecular species could be identified by electron microscopy, this would provide a means of identifying CEA, independent of the original one using antisera. This, in turn, would aid in assessing the purity of CEA preparations, and would provide an accurate procedure for monitoring new methods of purification of CEA.

Dr. Slayter and his collaborators recently reported that the structure of CEA, as visualized by the electron microscope, is quite distinct from that of other similar glycoprotein molecules they have studied, such as the blood group substances A and B, and cell-surface glycoproteins from mouse mammary tumors. CEA particles are short and thick, and appear in electron micrographs as distinctive rods or cruller-shaped particles with the dimensions of 9 × 41 nanometers (nm), when prepared from neutral solutions. When CEA is prepared from acid solutions, the molecule appears shorter, 10 × 36 nanometers.



EM studies of carcinoembryonic antigen molecules seek to enhance purification of the molecules and the development of highly sensitive antisera for greater accuracy in disease diagnosis. These electronmicrographs accompanied by diagramatic interpretations reveal a partially helical core structure, probably composed mostly of protein, with numerous protruding carbohydrate side chains. Magnification X245,000

Both direct electron microscopic observation, and the behavior of CEA in response to chemical modifications, suggest to Dr. Slayter's group that the molecule is folded into a partially helical core, probably composed mostly of protein.

From this core, numerous carbohydrate side chains protrude, contributing to the cruller-like appearance.

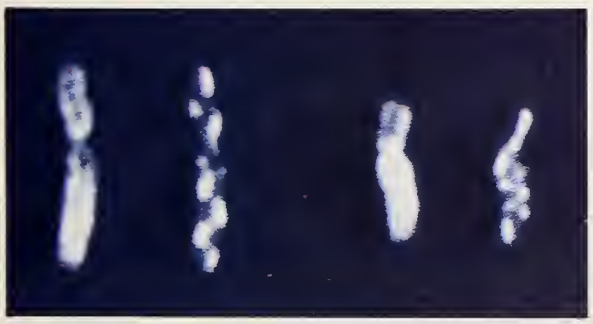
Dr. Slayter's laboratory now is exploring techniques for further chemical purification of CEA using electron microscopy as an additional criterion for purity of the fractions. This is complemented by collaboration with other groups performing detailed chemical and immunochemical analyses of CEA, which should reveal added clues as to the exact three-dimensional structure of the molecule. They are also testing whether antisera, prepared to the recently purified carcinoembryonic antigen, might allow greater sensitivity in the radioimmunoassay system for use in clinical diagnosis.

### Detection of Damage and Repair of Chromosomes

There is currently much concern about chromosome damage caused by a number of environmental agents, including pollutants, industrial chemicals, and drugs. The damage may mean that the exposed individual has an increased probability of developing cancer; or the effects may extend over several generations, because of heritable defects induced by exposure to these agents.

There are, in addition, a number of diseases which predispose toward increased chromosome fragility, and, frequently, result in neoplasms. Thus, the development of better methods to detect chromosomal damage is an important first step toward a better understanding of the underlying causes and processes. This should help to increase our ability to detect and assess the health hazard of various chemicals.

Chromosome breaks occur regularly as a result of wear and tear on the cell. Their incidence may be increased by exposure to hazardous agents. These breaks can be repaired by special enzyme systems which rejoin broken strands of DNA in the chromosomes. Since the DNA strands in each chromosome are paired, this repair process may result in an exchange of material between opposite strands of a DNA pair, the "sister-chromatids," which can be seen during the process of mitosis. A technique recently developed by a grantee of both NIGMS and National Institute of Child Health and Human Development (Dr. Samuel Latt, Harvard Medical School) permits visualization of sister-chromatid exchanges, and is therefore a sensitive indicator of possible genetic damage in human chromosomes. Such exchanges are relatively frequent, compared to permanent chromosome breaks, which makes it possible to get such information by examination of only a few cells. Agents previously examined for their ability to cause permanent chromosome changes can now be reexamined for their ability to alter the frequency of sister-chromatid exchanges.



Dark and light areas in the second and fourth chromosomes illustrate numerous drug-induced exchanges of sister chromatid DNA following exposure of cell to mitomycin C, exposure to BrdU, and staining with 33258 Hoechst fluorescent dye. By contrast, the same two chromosomes, stained with quinacrine, are uniformly fluorescent.

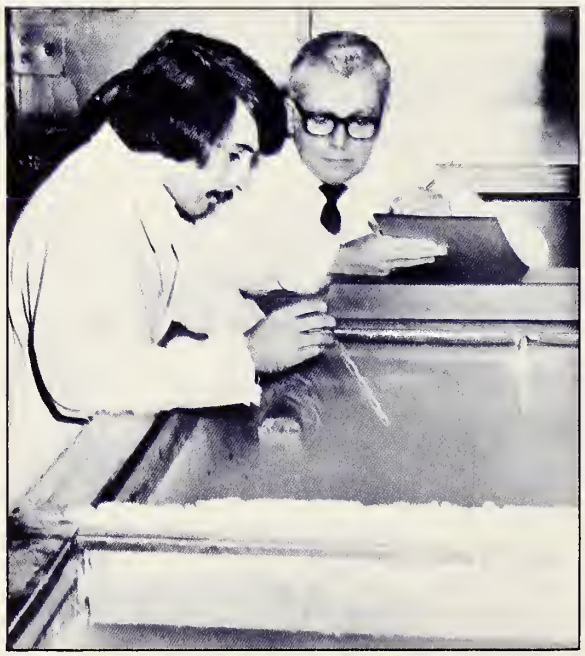
Dr. Latt's technique depends on the fact that the fluorescence of a chromosome stain, 33258 Hoechst, is markedly reduced after the cells are grown in the presence of the nucleic acid base analogue, BrdU. After growth in tissue culture medium containing the analogue, the stained chromosomes fluoresce only one-quarter as much as originally. Normally, the reduced fluorescence is localized on one of the two sister-chromatids. Sharp reciprocal alterations in fluorescence along the length of the chromosome are an indication of sister-chromatid exchanges.

### Human Cell Bank

The intact human being is far from an ideal subject of genetic studies. Traditional methods such as controlled breeding are not possible. In addition, a generation time of 20-30 years is hardly convenient, even for the most patient researcher. Thus, the student of human genetics has until recently had to rely on family pedigrees in order to obtain clues about the detailed mechanisms of human inheritance. It is therefore not surprising that we know far less about our own genes than those of the common bacterium *E. coli* or those of the laboratory mouse. Advances in tissue culture are now promising to provide a powerful aid in the study of human genetics. The new field of somatic cell genetics offers an approach to the "mapping" of human genes via tissue culture techniques.

Advances in the art of tissue culture have had other important effects on the study of inherited human diseases. We can now study many such diseases in cells derived from patients. In the past few years, the specific molecular defect of several genetic diseases has been identified through tissue culture methods. Furthermore, new strategies for therapy can often be explored and developed through the use of tissue culture, thus preventing needless trauma to patients. In recognition of the increased importance of these new developments to the study of human genetic disease, the National Institute of General Medical Sciences is supporting a repository of living human cells in tissue culture at the Institute for Medical Research in Camden, New Jersey. The repository distributes such cultures to the scientific community, on request, in order to facilitate research and teaching in human genetics.





Ampules of viable human mutant cells are maintained in liquid nitrogen storage at the Institute for Medical Research, Camden, New Jersey, awaiting retrieval and shipment to scientific users on request.

Now beginning its fourth year of operation, the facility is serving as an important stimulus to the advance of clinical genetics research.

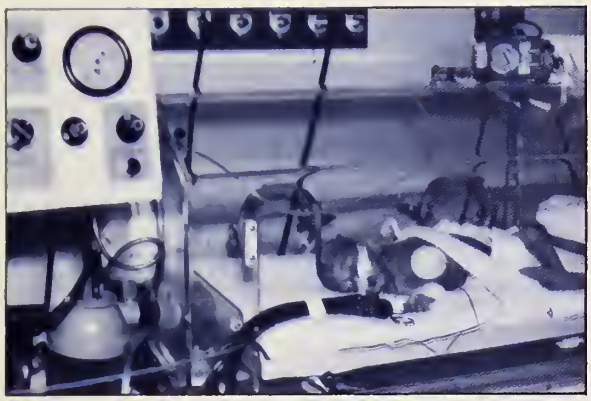
The most recent catalog lists more than 300 different cell cultures which are available for distribution. The list represents single-gene defects, chromosome aberrations, normal control cell lines, and some animal cell lines used in gene mapping studies. With the continued growth in the size and utility of the collection, the number of shipments to users is increasing. More than half of the shipments are to users in academic institutions, with the remainder to nonprofit institutions (23 percent), government laboratories (16 percent), and profit-making institutions (10 percent). About 10 percent of the shipments are made to laboratories abroad, including Canada, West Germany, England, France, Sweden, and Australia.

### Oxygen Toxicity Studies

While oxygen is essential to life, this important element can also present problems of extreme toxicity. The enzyme, superoxide dismutase (SOD), is receiving increasing attention for the role it may play in the toxicology of oxygen. This enzyme was discovered by Dr. Irwin Fridovich of Duke University, Durham, North Carolina, and really refers to a class of similar enzymes found in many different species and tissues. The enzyme appears to scavenge superoxide ($O^{2-}$) radicals from solution by converting them to hydrogen peroxide which is rapidly removed from the system by ubiquitous catalases and peroxidases. Superoxide radicals are a by-product of many biological oxidations and reactions with oxygen, and may also be formed in tissue by the action of ionizing radiation.

Dr. Fridovich's work has led to the characterization of three quite different superoxide dismutase enzymes: a copper-zinc containing protein found in cytosol and red blood cells; an iron-containing protein (from the periplasmic space of bacteria); and a manganese-containing protein (found in the matrix space of bacteria and in mitochondria). Work is continuing to determine the mechanism of oxygen toxicity in many organisms and the details of the manner in which SOD prevents or reduces this toxicity.

Because of its involvement with oxygen metabolism, SOD is also receiving attention from investigators interested in the lung. While this enzyme is present wherever oxygen is needed, used, and abundant, its role in the development of the lung and its ability to function in certain disease states are being evaluated by Drs. Anne Autor, Lee Frank, and Robert Roberts at The Toxicology Center, and Department of Pediatrics, University of Iowa. Hyaline membrane disease (respiratory distress syndrome) is a well-known cause of mortality in the premature infant and is, perhaps, the most difficult disease to handle in the newborn. Drs. Autor, Frank, and Roberts have found that the SOD level in the lungs of infants with fatal hyaline membrane



disease was significantly lower than the activity of the enzyme in normal adult lungs. Furthermore, they found that the SOD activity in the blood of infants with broncho-pulmonary dysplasia (a complication associated with prolonged oxygen therapy) was also low when compared to normal adult blood activity.

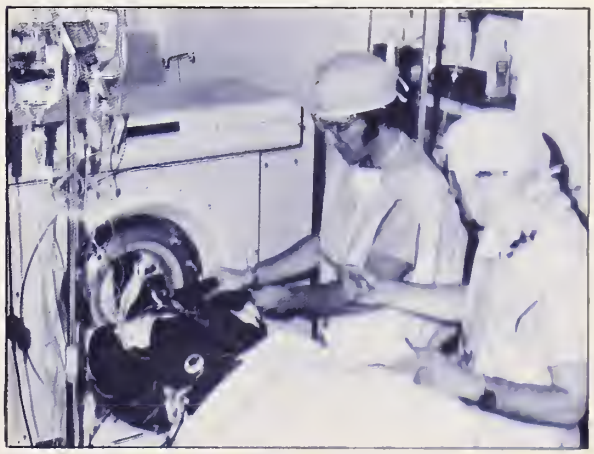
The effect of high oxygen tensions on lung tissue, studied in the laboratory, shows that lung tissue from very young rats, when incubated in plasma in the presence of 100 percent oxygen, nearly doubles its SOD activity. In premature infants, or those with respiratory distress syndrome, it appears that due to immaturity, increase of the protective enzyme, SOD, is retarded and the lungs may be seriously damaged by metabolites of oxygen. Because this enzyme acts by removing highly reactive oxygen radicals formed in the body in the presence of oxygen, the observation of diminished SOD activity associated with lung immaturity has important implications for the treatment of infants with hyaline membrane disease. These infants, who have difficulty breathing, require treatment with oxygen. Now it appears that one source of their respiratory difficulty may be an inability to handle the products of oxygen metabolism. These studies may provide the basis for improved therapy for hyaline membrane disease.

### New Therapy for Head Trauma

Head injury is one of the leading killers following severe trauma. Recently a team of investigators at the Universities of Washington and Pennslyvania anesthesiology research centers, supported by NIGMS, successfully treated 12 patients in coma resulting from head trauma, by using a combination of barbiturates and hypothermia (lowering of body temperature).

For some time, it has been known that induction of hypothermia or administration of barbiturates reduce the blood flow to, and metabolic rate of, the brain and reduce intracranial pressure (ICP), thereby lessening the harmful effects on individual cells resulting from head trauma. Studies by these investigators on animals (and a few patients with elevated ICP) have shown that barbiturates can reduce ICP within 0.5 to 3 minutes, but engender risk of cardiovascular depression when used in the high doses required. Hypothermia below 27°C can also reduce intracranial pressure, but causes cardiovascular instability and possible ventricular fibrillation of the heart. By using a combination of moderate doses of barbiturates and mild hypothermia (30°C), ICP could be reduced to the desired extent without these side effects. Further, the combined use of barbiturates and mild hypothermia achieved a significantly greater reduction in oxygen and glucose consumption by brain tissue (about 70 percent) than either method alone. The animal experiments with combined treatment were considered promising enough to test this mode of therapy clinically.

The investigators reported the successful treatment of 12 comatose patients with head trauma having a poor prognosis. The patients had had persistent intracranial hypertension, despite intensive therapy with mechanical ventilators, steroids, and diuretics. This therapy was continued while barbiturates were added and serum levels of pentobarbital maintained between 2.7 and 3.3 mgm percent. This was followed by induction of hypothermia, by wrapping the patient in special water-cooled blankets which lowered the body temperature to 30°C. Several of the physiologic and metabolic changes, including the alterations of ICP previously seen in animals, could be confirmed in the patients.



Anesthesiologists care for head-injured patient during computerized axial tomography (CAT) scan

The investigators observed that barbiturates alone significantly reduced ICP. However, when hypothermia was added, there were further reductions in ICP. No increase in cardiac irritability or other untoward effects were observed. The most effective treatment span was found to be in the range of 4-5 days. The patients receiving such treatment made good recoveries in mental acuity, but some limitations remained in their motor activity. Further studies are needed to confirm these beneficial effects, but for the first time it may now be possible for some patients with devastating neurological injuries to make remarkable recoveries, and lead useful lives.



### Miniature Fiberscopes

To examine body cavities without surgical exploration an endoscope is usually employed. In recent years a fiberoptic technique has been developed in which light is transmitted for the endoscopy through a bundle of glass fibers.

Fiberoptic endoscopes consist of a fiberoptic imaging structure for viewing, a channel for performing biopsy, aspiration or insufflation, and a bundle of optical fibers to transmit light. Smaller endoscopes are now needed, which means it is necessary to reduce the size of these component parts. With small fiberscopes now in use, however, the light-transmitting optical fibers occupy nearly one-half of the cross section of the instrument.

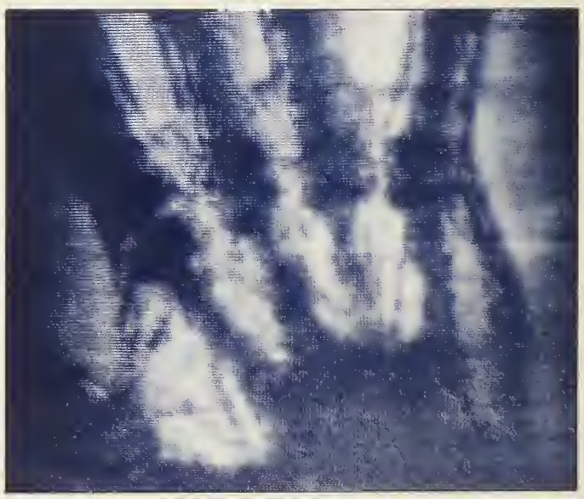
In these instruments the source of light for illumination is a projector or an arc lamp which is imaged onto the bundle of optical fibers. Since such light sources are incoherent, they cannot be focused to a very small cross section. With coherent light sources the light beam can be focused to the diameter of a single fiber. Such light can be obtained by using lasers, but a major drawback until now has been the unavailability of the multicolor lasers that are required to synthesize white light.

A technique was recently developed by Dr. Max Epstein at Northwestern University's Fiber Optics Research Laboratory in which a three-color output from an ion laser is passed through a single optical fiber. A krypton ion laser was used with a special broad band output mirror to obtain simultaneous output of three different colors—blue, green, and red. The resulting light was a white light beam easily focused onto a single optical fiber. At the end of the flexible 1 meter fiber the output light can illuminate an 8 inch × 8 inch area, and it is adequate for viewing and color photography. This work will undoubtedly be significant for the future design of endoscopic and related medical instruments.

### Ultrasonic Camera for Viewing Tissue Structures

The use of ultrasound for visualizing internal organs is now well established in diagnostic medicine. The images made with currently available instruments show cross sections of the anatomy—quite different from the view presented with X-rays. Although these so-called "echograms" are useful in obstetrics and for demonstrating the outlines of certain abdominal organs, it became apparent several years ago that—with further research and development—ultrasound could also be used to form images that would bear a close resemblance to the X-ray image, thus providing a wider range of diagnostic applications. Such an "ultrasonic camera" has how been developed at the Stanford Research Institute (SRI).

The SRI ultrasonic camera operates in a manner similar to that of a television camera, but with sound waves rather than light waves. The high-frequency waves—100 to 200 times greater than the highest audible pitch—are directed into the body, where they are bent, absorbed, and reflected by the tissue. The waves emerging from the body are collected and brought into focus by an ultrasonic lens system, thus forming an image of the tissue structures. The images reveal bones, tendons, cartilage, blood vessels, muscle, and various organs. A physician can focus at a desired depth in the body and observe the organs in motion as the patient breathes or moves or as the organs are palpated.



Ultrasonic visualization of the internal structure of the hand provides an excellent image of the bones, joints, tendons, ligaments, and vascular structures.

The results of initial tests of the camera are very encouraging. Ongoing research promises to improve the image quality still further, and significant diagnostic applications are anticipated in abdominal imaging, obstetrics, pediatrics, and orthopedics.

In general, ultrasound has two major advantages over X-rays in the noninvasive visualization of internal body structures. First, it is capable of visualizing soft tissues of the body in as well as the bony structures, whereas X-rays are limited to the visualization of only the hard, bony tissues of the body unless special measures are taken, such as the injection of dyes to permit viewing of soft tissues, which in some patients can be hazardous. Second, while X-rays are known to cause cellular and tissue damage, ultrasound, according to available data, appears to be completely safe at the levels of intensivity used in existing diagnostic equipment. Thus, ultrasound is widely used today in obstetrics, for visualization of the fetus, where X-rays ordinarily are considered much too dangerous.

Advances in microsurgical techniques and technology have enabled scientists to understand more clearly the nature of the human brain and the diseases which affect it.



## Brain Scanning by Computerized Axial Tomography

Diagnosis and patient monitoring in clinical neurology and neurosurgery have been revolutionized by the recent development of a non-invasive brain scanning technique known as computerized axial tomography, or CAT. Originally conceived of by Dr. William Oldendorf at UCLA and brought to clinical feasibility by Godfrey Hounsfield of EMI, Ltd. in England, the technique combines rapid scanning of the head by a narrow X-ray beam coupled with a computer which translates the differences in tissue density detected along the beam path into numerical or pictorial displays. The technique is approximately 100 times more sensitive than conventional X-ray procedures. It provides far greater resolution of the various brain structures and eliminates the need for some of the traditional X-ray methods, with their attendant risks and discomfort.

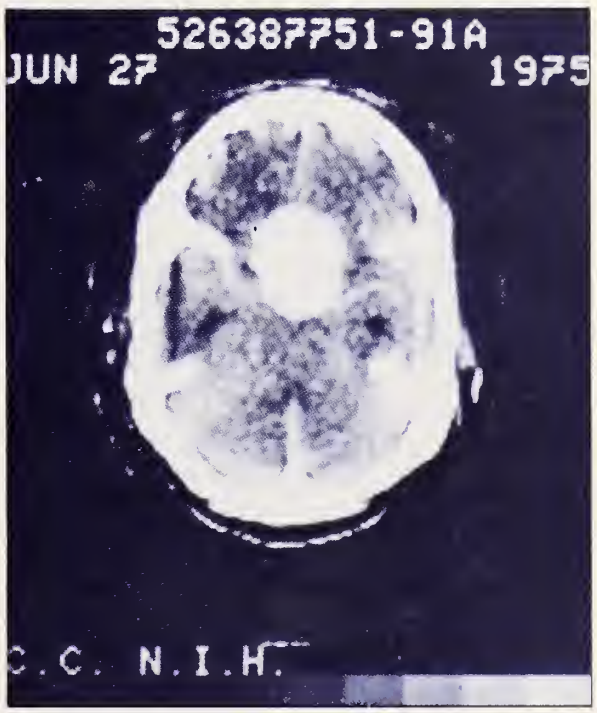
In the new CAT technique, a pencil-thin beam from the X-ray source passes through the head or body to be picked up by crystal detectors on the opposite side. Both source and detectors are fixed in a moving circular frame that permits the X-ray beam to rapidly scan the head or body in a series of "slices" or cross-sections. The signals from the detectors are fed into the computer which instantly calculates for each of the 25,600 points along the total scan the differences between the emitted X-rays and those received by the detectors. The differences reflect the absorption coefficients of intervening bone, tissues, blood, and spinal fluid.

The brain can be completely scanned in less than 5 minutes, but the range of normal and abnormal anatomical features that can be distinguished is striking. The technique is sensitive enough to detect differences in absorption (or density) of only one unit on a scale from -500 (air) to 500 + (bone). The computer can generate oscilloscopic images, like TV-pictures. These reproduce the cross-sections of brain tissue structures as if the brain itself had been laid open at each successive level for viewing. While the scan is in progress, the neuroradiologist and the patient's physician can follow the scanned section on the screen and can enhance or suppress certain portions of the image to bring out specific details.

In the head, the fluid spaces, the gray and white matter, and certain landmarks like the pineal gland and choroid plexuses are readily distinguished by CAT but seldom by conventional X-rays. Tumors, hemorrhage, brain atrophy and edema are among the pathological conditions that are quickly, easily, and more consistently visualized by CAT than by other neuroradiological techniques. Hence it is not surprising that each CAT is in use, literally day and night.

The technique has already been extended to problems in the eye, where tumors and other masses in the bony orbit behind the eye have been notoriously difficult to diagnose. CAT is also being used for other parts of the body. Dr. Giovanni Di Chiro, National Institute of Neurological and Communicative Disorders and






Round, central, white density represents tumor (meningioma) at base of brain

Stroke, and his co-workers have pioneered the use of the system to study the spinal cord and its disorders. The prototype of a whole body scanner has been developed to permit scanning of heart, lungs, and abdominal organs, where an even more rapid scan time helps minimize artifacts from heart beat and respiration. The faster scanning will also prove useful for the unconscious or disoriented patient where movement makes brain scanning somewhat diffficult. CAT has also produced surprising results in patients with epilepsy. At the Mayo Clinic, Dr. Hillier Baker and colleagues have detected a number of unsuspected brain lesions (tumors, atrophy, etc.) in a large series of seizure patients.

CAT may also be used to monitor stroke patients

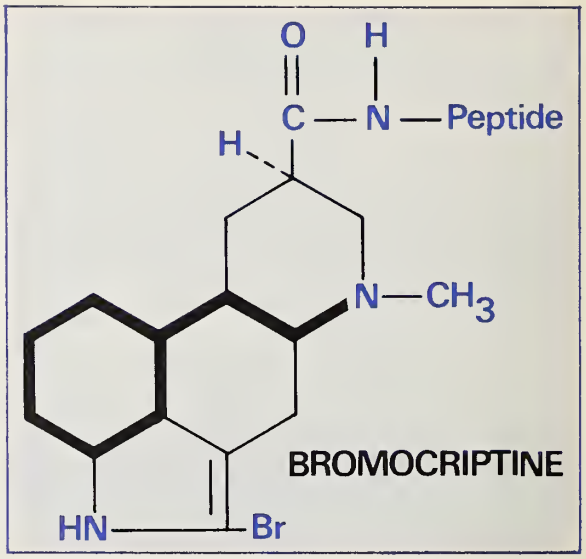
during the acute phase and subsequent stabilization, and to evaluate effects of chemotherapy for brain tumors. Dr. Juan Taveras and colleagues at the Massachusetts General Hospital have shown that the combined use of CAT and contrast angiography provide detailed visualization of brain vessels and their abnormalities never before achieved by conventional angiographic techniques. It is anticipated that it will be possible shortly to use the brain scanner to detect radioisotopic compounds injected into the patient and hence evaluate the functional state of the brain. Thus computerized axial tomography will continue to have an extraordinary impact on diagnostic radiology and clinical management, especially in the neurological and communicative disorders.



### New Drugs for Parkinsonism

A new approach to the therapy of Parkinson's disease—utilizing drugs known as dopamine receptor agonists—has been reported by NINCDS scientists, Drs. Donald B. Calne, Ronald Kartzinel, and Ira Shoulson. One such drug, *bromocriptine,* has produced an excellent response in double blind studies with patients at the NIH Clinical Center. *Bromocriptine* works more directly than levodopa, which in the past 5 years has become the mainstay of antiparkinson treatment. The scientists feel that success with the new drug is the most promising development since levodopa was introduced.

Progress in the drug treatment of parkinsonism has been one of the major neurological success stories of the decade. For the better part of a century, these patients were treated with extracts from the belladonna plant. In the 1950's experimentation with tranquilizers as a means of controlling high blood pressure produced a large amount of new information on the action of chemical neurotransmitters. Contributions came from a number of countries, but some of the most crucial basic research was done at the National Institutes of Health. First, reserpine, a tranquilizing drug, was found to produce Parkinson-like symptoms when given in heavy doses. It was also shown to reduce the high concentrations of the neurotransmitter chemical, dopamine, in parts of the brain responsible for motor control. In 1960 scientists at the University of Vienna found a striking shortage of dopamine in the brains of Parkinson patients at autopsy. This important finding clearly pointed to the possibility of replacement therapy, and led to immediate efforts to treat Parkinson's disease with dopamine.




Early attempts to replace dopamine directly (intravenously) were unsuccessful. It would not cross the blood-brain barrier—a protective biochemical mechanism which selectively screens substances passing from the blood into the central nervous system. However, scientists found that dopamine's metabolic precursor—the preceding link in the chain of chemical reactions leading to its production—would cross the barrier.

At first the precursor, levodopa (L-Dopa) was not given in large enough dosage, for a long enough time, or by the best route to build up an effective and stable blood level. Then it was found that most patients could tolerate effective amounts by starting them on small oral doses and slowly increasing the dose. Extensive trials led to the approval of levodopa for prescription sale in June 1970.

The new therapy, however, was not without problems. Chief of these was the side effects caused by the extremely large doses necessary to control parkinsonism symptoms. Levodopa is broken down very rapidly in the body, and large doses are necessary to achieve the saturation required for penetration into the brain. In some cases, the side effects were so severe that the drug had to be discontinued. A way was found to reduce the high dose levels—and the side effects—by giving levodopa with an enzyme inhibitor, a substance which slows its metabolic breakdown. Still, many patients continue to have severe problems.

The NINCDS scientists believe that one factor contributing to the failure of patients to respond well to levodopa is the reduced brain concentra-

tion of the enzyme which converts it to dopamine: L-aromatic amino acid decarboxylase. Another possible source of trouble is the production of active metabolites of levodopa other than dopamine. For example, norepinephrine may be responsible for some of the unwanted reactions. Both of these drawbacks, the researchers have predicted, can be overcome if drugs can be found which enter the brain and act directly upon dopaminergic receptor sites, i.e., dopamine agonists. Such an agent could still operate when the enzymic machinery required to convert levodopa to dopamine has been destroyed. Also, if the drug were sufficiently specific for dopaminergic receptors, adverse effects would not be generated by activation of other synapses. So far, *bromocriptine* seems to meet these conditions. Adverse reactions are dose dependent, reversible, and qualitatively similar to those encountered with levodopa. In one series of patients, the majority were able to stop levodopa while taking *bromocriptine*. In another, the drug was added to otherwise optimum therapy, including levodopa. The improvement was measured at about 10 percent in patients with minor disabilities, and about 20 percent in those with more severe disease.

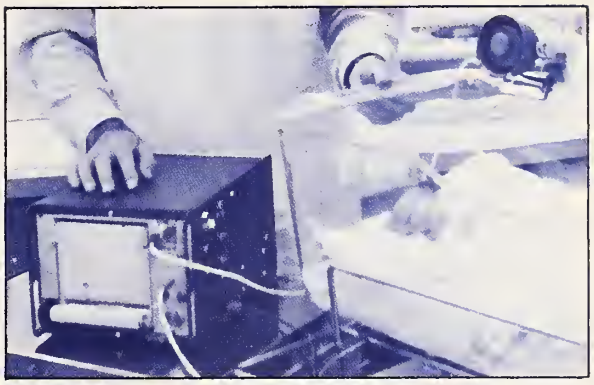
### Hearing Test for Newborns

A new automated test to detect hearing impairments in newborns may help save thousands of children from a life of silence.

Physicians, hearing and speech specialists, and educators have long been interested in identifying children with hearing impairments as early as possible. If the problem can be treated medically, serious damage to the hearing mechanism may be averted. If treatment is not possible, the child may be fitted with a hearing aid and education of both the child and the parents can be started at the beginning of the child's language learning years.

The "crib-o-gram" system, which was developed at Stanford University School of Medicine by NINCDS-supported scientists F. Blair Simmons and Frederica M. Russ, works on the principle that babies in a relatively quiet condition will respond to loud sound by some startled movement whereas babies in an active state will often respond by becoming suddenly quiet.

In crib-o-gram testing, a number of moderately loud noises are presented over a 24-hour period and the infant's resulting movement is recorded on a strip chart. The record is then scored for



Crib-O-gram

presence or absence of response according to specific criteria and a child either passes or fails the test.

Six months after hospital discharge, babies who failed the crib-o-gram screening test are given a different screening test and children who fail that procedure are referred for additional audiologic workup. All children who have passed the crib-o-gram test are also followed up by questionnaire at the age of 2 years in order to determine whether any deaf children were missed by the crib-o-gram test.

The instrumentation consists of a sensitive motion detector placed either under the plastic bassinet, standard plastic nursery crib or under the mattress of an isolet in an Intensive Care Unit (ICU). Information received by the detector is fed to a multi-channel strip chart recorder (usually located in another room) via wall plugs in the nursery. Eight cribs within the range of the same speaker may be simultaneously recorded and a switching network allows the testing to progress from room to room; if desirable, a single recording system and switching network could test over 100 babies each day. The record for each crib is identified by a digitally-coded circuit.

Nonprofessionals can be trained to score the strip chart records accurately. However, inclusion of silent tests and periodic independent record scoring by a professional are recommended. Research is now under way to improve the criteria for scoring the recordings to reduce the number of false positive responses.

Crib-o-gram testing appears to be especially useful for high risk children and preliminary results indicate that a larger number of deaf children are identified within the ICU population. Studies are now under way in other hospitals to determine the feasibility of crib-o-gram testing for other institutions.

## Motion Restored by Experimental Device

A 22-year-old man who was paralyzed by an upper spinal cord injury 2 years ago is now a freshman at Kent State University. He is able to use his hand and arm to print, type, answer the phone, and drink from a cup.

He is one of 14 spinal cord patients testing one of the most promising experimental electrical devices to date for regaining lost function. The device was developed by Dr. Thomas Mortimer and colleagues at Case Western Reserve University in Ohio. The Case team is part of an NINCDS contract program directed by Drs. Karl Frank and Frederick Hambrecht, investigating possibilities of developing sensory prostheses (aids) for the blind and the deaf in addition to the paralyzed.



Bypassing lost neural function

The three-part device being tested by the Kent State student and the 13 other patients is connected by wires concealed under their clothing. It consists of a transducer, attached to their chest; a stimulator, placed under the wheelchair; and tiny stainless steel electrodes, about one-third as thick as a strand of hair, implanted in their hand muscles. A major advantage of the system is that surgery is not required to implant these electrodes. Instead, the wires are coiled into a helix (like a door spring) and placed inside a hypodermic with just the tip of the wire extending beyond the hypodermic needle. When the needle is inserted, the wire then catches in the muscle and is implanted.

Another advantage of the system is that patients initiate their hand and arm motion themselves by moving their shoulder muscles, usually left intact in upper spinal cord injury, toward the center of their chest. The degree of movement is picked up by a beam (part of the transducer) attached to the chest. The transducer then signals the stimulator which in turn activates the implanted electrodes to produce muscle contractions for grasping and moving.

Learning to use the device is a hard and tedious step-by-step process. First, electrodes are inserted, and if the patient's hand and arm muscles are weak from disuse, they are strengthened over a period of several weeks by eliciting muscle contraction through electrical stimulation. Once sufficient muscle strength has been restored, detailed studies on artificial control—tailored to the individual—are begun. These include selecting the ideal electrodes, noting muscle responses to various current levels, and determining the fatigue properties of each patient's muscles. Finally, the patient is taught how to control electrode stimulation using shoulder motion.

Doctors point out that the system still has significant problems. For instance, electrodes migrate within the muscles. So doctors have to make frequent checks to determine when the electrodes are no longer exactly in place, and then remove them and insert new ones.

The scientists are also working on a feedback system which would enable patients to determine the force of their grasp without having to look. In the rudimentary feedback system being developed, the muscle stimulating current artifically generates sound which differs depending on the force of stimulation. Research at NINCDS by Drs. William Marks and Edward Schmidt, who are recording from cells in peripheral nerves (those innervating muscles) and in the brain's cerebral cortex, is unraveling some of the intricate and complex circuitry of the neural feedback systems which provide communication between nerve and muscle.

Scientists are optimistic that the device can be refined enough to become generally available to upper spinal cord patients who would have the tremendous boost of being able once again to perform some tasks for themselves.

## Freeze-Fracture Opens View of Protein's World

Spectacular panoramic views of split-open protein membranes, made possible by an exciting laboratory technique called "freeze-fracture," are providing a whole new perspective on how proteins abet the growth and spread of nervous system viruses.

Through freeze-fracture, Drs. Thomas S. Reese and Monique Dubois-Dalcq have seen the growth and spread of the incipient panencephalitis virus, which produces a devasting and fatal brain disease in children and young adults. They are now applying the technique to the study of other nervous system viruses.

In addition, Dr. Reese and his NINCDS colleagues, who are among the first in this country to apply freeze-fracture to nervous system studies, have also characterized proteins which act as "receptors" for the chemical messages conveyed from one nerve cell to its neighbor. Receptor destruction in muscles is thought to cause the weakness seen in disorders like myasthenia gravis.

Why can these unique views be obtained only by freeze-fracture? First, tissue can be examined closer to its natural state since it is frozen and needs no chemical preservatives; and second, by splitting the membrane and producing an unobstructed view in every direction, the size and distribution of proteins as small as one ten millionth of an inch in diameter can be scrutinized. This information on protein size and distribution—which, significantly, determines many of the characteristics of nerve cells—cannot currently be obtained in any other way.

In the viral studies, Drs. Reese and Dubois-Dalcq found that viral genetic material attached to proteins in the membranes which project from the cell surface. These are the proteins which enable cells to stick together when they come in contact. The viral attachments then organize and deform the normal cell membrane into viral buds, covered with coats of viral proteins. Whereupon, it is viral proteins which project from the cell surface, enabling the virus to stick to and infect other cells. Studies are underway to determine whether other viruses which attack the nervous system form and spread in the same way.

In addition to their role in viruses, proteins are also suspect in disorders such as myasthenia gravis, where there are defects or changes in cell "receptors." These protein receptors convey messages which are passed from cell to cell by small packets of chemicals called neurotransmitters, at the synapse, or junction between the two cells. Scientists have long known that neurotransmitters activate the receptor of the neighboring cell to produce a message. But not until freeze-fracture did they discover that the size and distribution of receptors depend on whether they characteristically produce excitatory or inhibitory chemical messages when acted on by a transmitter. Drs. Reese and his colleagues, Drs. Dennis Landis and John Heuser, expect their findings may help determine what may go wrong—and how it could be prevented—in receptors implicated in myasthenia gravis and related disorders.




Panencephalitis virus (SSPE) budding from a cell in tissue culture. The arrow points to a virus which has formed from the bud. The tiny particles in the membrane of the bud at A and B differentiate it from a normal cell membrane. Magnification X45,000

Excitatory synapse near the surface of the cerebellum prepared by freeze-fracturing. Particles on the membrane of the synapse are receptors which produce an excitatory effect in the nerve cell membrane by a transmitter chemical. Magnification X75,000

## New Clues Link Myasthenia Gravis to Immune Disorder

NINCDS-supported researchers have produced evidence that the debilitating neuromuscular disease myasthenia gravis (MG) is an autoimmune disorder in which the victim's own body sets up an immune reaction against itself.

Myasthenia gravis is characterized by progressive muscular weakness of the arms, legs, face, throat and chest. Recent research on MG has focused on the neuromuscular junctions—the point at which the motor nerve endings transmit their signals to the muscle fibers.

Normally a substance called acetylcholine is released from the nerve ending and crosses a gap or synapse to a muscle region called the end plate. There the acetylcholine attaches to certain protein molecules on the endplate known as receptors and triggers muscle contraction. In MG this mechanism is impaired.



The recent findings indicate that the myasthenic's lymphocytes—the white blood cells that attack invading germs—interfere with the receptor molecules on the muscle end plate.

Drs. Jim Patrick and Jon Lindstrom of the Salk Institute in La Jolla, California, were able to induce myasthenia-like weakness in rabbits by immunizing the animals with acetylcholine receptor (AChR) protein purified from the electric organ of the eel.

The antibodies raised in the rabbits to ward off the foreign electric eel AChR protein reacted with the animal's own receptor protein and produced progressive weakness similar to that observed in myasthenia gravis. Also, the muscle end plates of the affected rabbits resembled those of a myasthenia victim.

These results, which have been confirmed in several other laboratories and have been extended to other animal species, represent a promising new approach for studying the mechanisms involved in the cause and cure of myasthenia gravis.

The animal model has generated an intensive search for an immunologic cause of MG.

A team of researchers headed by Dr. Obed Abramsky of Israel's Weizmann Institute of Science, has found that a mixture of proteins from normal muscle were attacked in the test tube by lymphocytes from myasthenia patients. Drs. Barry G. W. Arnason and David P. Richman of Boston's Massachusetts General Hospital have shown that the lymphocytes seem to be specifically targeted on the receptors. Lymphocytes taken from myasthenics attacked and destroyed purified receptor extracts.

NIH scientists Drs. Adam N. Bender, Steven Ringel and W. King Engel, utilizing a new molecular probe, have shown that sera from a majority of patients with MG contain an immunoglobulin (probably an antibody) that

Rat on right exhibits characteristic myasthenic weakness following immunization with receptor protein.

binds to the receptor. The investigators believe that this is likely to be the circulating factor causing the weakness in myasthenia gravis.

These immunologic clues offer hope that the mystery of myasthenia gravis will be unravelled and a more effective weapon against this disease will be developed.

### Genetic Implications of Multiple Sclerosis

Striking new evidence suggests that certain persons may inherit the tendency to develop multiple sclerosis—possibly by receiving genes which produce a defective immune (defense) system against invaders. If this lead is confirmed by future studies, it may become the pivot point around which other clues to this devastating neurological disorder fall into place.

Until now, scientists have had few concrete handles in studying multiple sclerosis (MS), known as the great crippler of young adults. Symptoms usually begin to appear in patients between their 20th and 40th years, and often include severe and sometimes incapacitating muscular weakness, dizziness, and blurred vision. The one common physiologic characteristic has been the as yet unexplained destruction of the myelin (fatty) sheath which surrounds and insulates nerve fibers in the brain.

Now a second physiologic characteristic may have been found. The recent flurry of genetic activity centers around what are known as "histocompatibility antigens," protein molecules which are present in all cells. Although everyone has histocompatibility antigens, individuals have different types depending on the inherited genes which direct their production. Important recent studies indicate a much higher incidence of three particular antigens among

patients with MS compared to the population at large. These antigens are types "HL-A3," "HL-A7," and "LD-7A."

Dr. Casper Jersild (formerly at the New York Sloan-Kettering Cancer Center and now in Copenhagen, Denmark), in a study of 1465 MS patients and 4238 normal controls recently found that about 40 percent of MS patients had the HL-A7 antigen as opposed to 25 percent with this antigen in the control population. But even more significantly, Dr. Jersild and colleagues found that 60 percent of the MS patients had the LD-7A antigen, compared to 18 percent in the control population. These studies indicate a dramatic association between MS and particularly the LD-7A antigen.

In addition, studies by Dr. Barry Arnason and colleagues at Harvard University have shown that the incidence of persons with the HL-A3 antigens seems to follow the peculiar geographic distribution of MS. Earlier studies found MS to be most prevalent in the temperate and colder climates of Western Europe, and North and South America, but exceedingly rare in the tropics. Similarly, Dr. Arnason's studies show the HL-A3 antigen is uncommon among the Japanese, where MS is rare; and, the antigen is more prominent among northern Europeans, where the incidence of MS is high, as compared to southern Europeans where it is lower.

Just how important the increased incidence of the antigens is in MS patients is not yet known. But, genes which produce them have been found in animal models to be closely linked to what are known as "immune response genes." These are the genes thought to be responsible for the body's defensive reaction against invaders. Therefore, if there are defects in the immune response genes, these defects may also show up in their closely linked neighbors, the HLA and LD genes whose markers are the HLA and LD antigens.

In addition to the genetic implications are virological findings. A number of studies, many supported by NINCDS, have shown that some patients with MS have increased levels of antibodies against measles virus in their blood and cerebrospinal fluid. Although no virus has yet been found to cause MS, it may be possible for a defective immune system to allow a virus to persist in the body for years before some triggering factor spurs it into action. Such "slow viruses" have been found by NINCDS scientists to cause some other neurological disorders, and could account for the late age of onset in MS.

Scientists hope that this information will ultimately lead to unraveling the complex interrelationship between the genetic, immunological and virological factors which seem to be involved in this baffling disorder.

### Animals Aid in Identifying Viral Causes of Birth Defects

This year NINCDS and collaborating scientists produced the first experimental primate models for the influenza virus and the Venezuelan equine encephalitis virus, and demonstrated that they may be capable of producing malformations in the human nervous system.

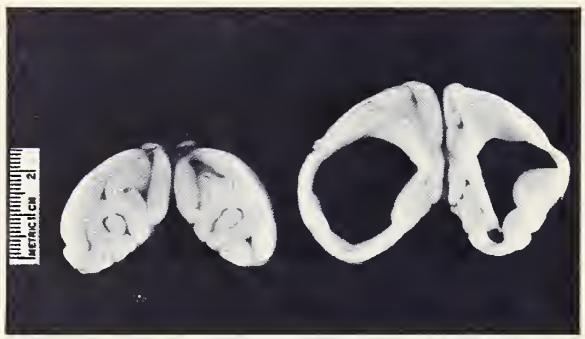
Understanding the role of viruses in causing birth defects depends to a very large extent on developing a good model for seeing and studying these tiniest of all parasites in action.

The role of the influenza virus in damaging the nervous system has long been suspected. However, an experimental primate model was needed to prove it. Such a model has now been produced by NINCDS scientists Drs. William T. London, David D. Fuccillo, and John L. Sever in collaboration with Dr. Stephen Kent, George Washington University, Washington, D.C.

Using pregnant rhesus monkeys, serologically negative for influenza virus antibody, these investigators injected influenza A virus directly into the rhesus fetus by inoculating through the intact uterine wall. This procedure was carried out at approximately 100 days gestation.

Two fetuses studied 3 and 11 days after inoculation showed the presence of virus in most of the tissues. This indicated that replication of the virus had occurred.

The other fetuses were delivered at term. Half of



Corresponding sections of brain of 1-month-old rhesus monkeys following *in utero* intracerebral inoculation of influenza A virus. Brain on left, inoculated with control material, appears normal: brain on right, inoculated with virus shows bilateral hydrocephalus.

the virus-inoculated animals had hydrocephalus, a condition characterized by an abnormal increase in the amount of fluid within the skull causing enlargement of the head and destruction of the brain. These findings suggest the possible importance of influenza virus infection in human pregnancies.

Similar studies using the vaccine strain of Venezuelan equine encephalitis virus were conducted by inoculating rhesus monkey fetuses at approximately 100 days gestation. At birth, almost all infected monkeys had cataracts and were later found to be blind. The animals also had enlarged heads due to hydrocephalus. This research, carried out by Drs. London and Sever in collaboration with Dr. Kent and Dr. Neal Levitte, Army Institute of Medical Research, Fort Dietrick, Maryland, constitutes important new evidence about the possible viral origin of birth defects.

### A One-Step Operation to Control Hydrocephalus

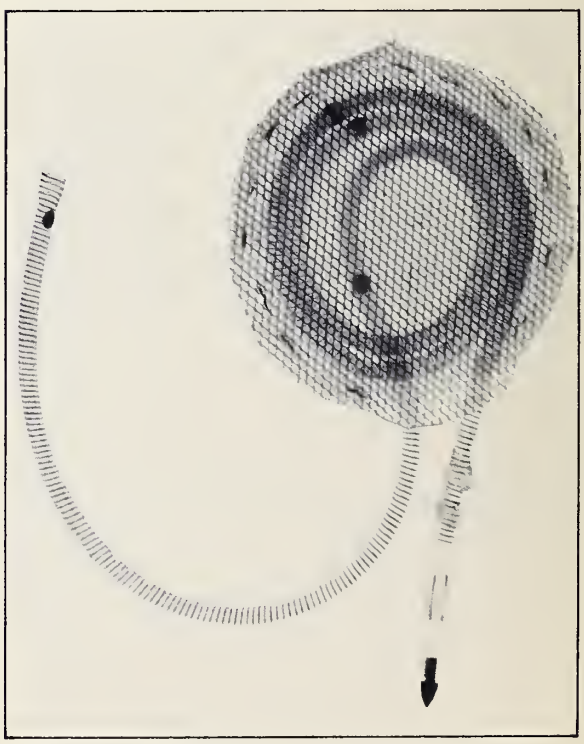
A new expanding shunt to help infants with hydrocephalus—sometimes called "water on the brain"—was used experimentally for the first time this year. It represents the only significant change in the classic procedure for draining off excess fluid from the brain in more than a quarter of a century.

The shunt was developed by collaborating scientists Dr. Thomas H. Milhorat, Children's Hospital, Washington, D.C.; Dr. J. E. McClenathen, George Washington University, Washington, D.C.; and Dr. Mary K. Hammock, National Institute of Neurological and Communicative Disorders and Stroke.

Hydrocephalus is one of the most common causes of mental retardation. It is estimated that one infant in 300 is born with the condition, usually as a result of a prenatal malformation or infection in the brain. Another one infant in 100 develops it following a childhood illness such as meningitis.

In hydrocephalus, the water usually present in the cavities of the brain is prevented from escaping due to an abnormal blockage of the exit avenues. As the fluid accumulates it causes the brain and skull to enlarge and destroys the brain substance. Often paralysis, blindness, mental retardation, lack of speech, or convulsions result.

Before the development of the original ventricular shunting operation for draining off excess fluid from the brain, most infants with hydrocephalus died by the end of their second year. Now this mechanical process makes it possible in some cases to preserve life and function for many years.



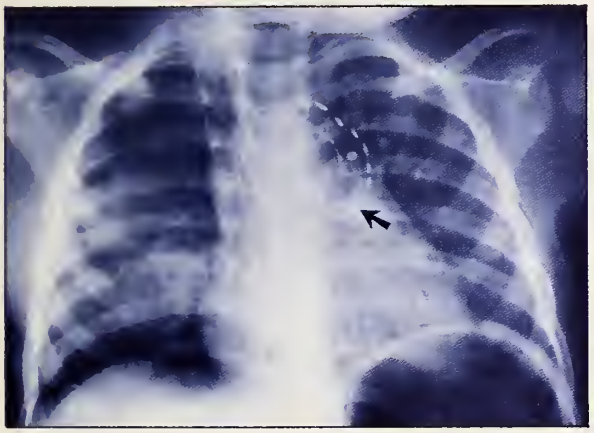
Prefabricated Silastic pouch containing extra coils of tubing. This pouch is positioned in the chest in the course of a shunt from the cerebral ventricles to the heart. There will be a progressive uncoiling of the tubing as the patient grows.

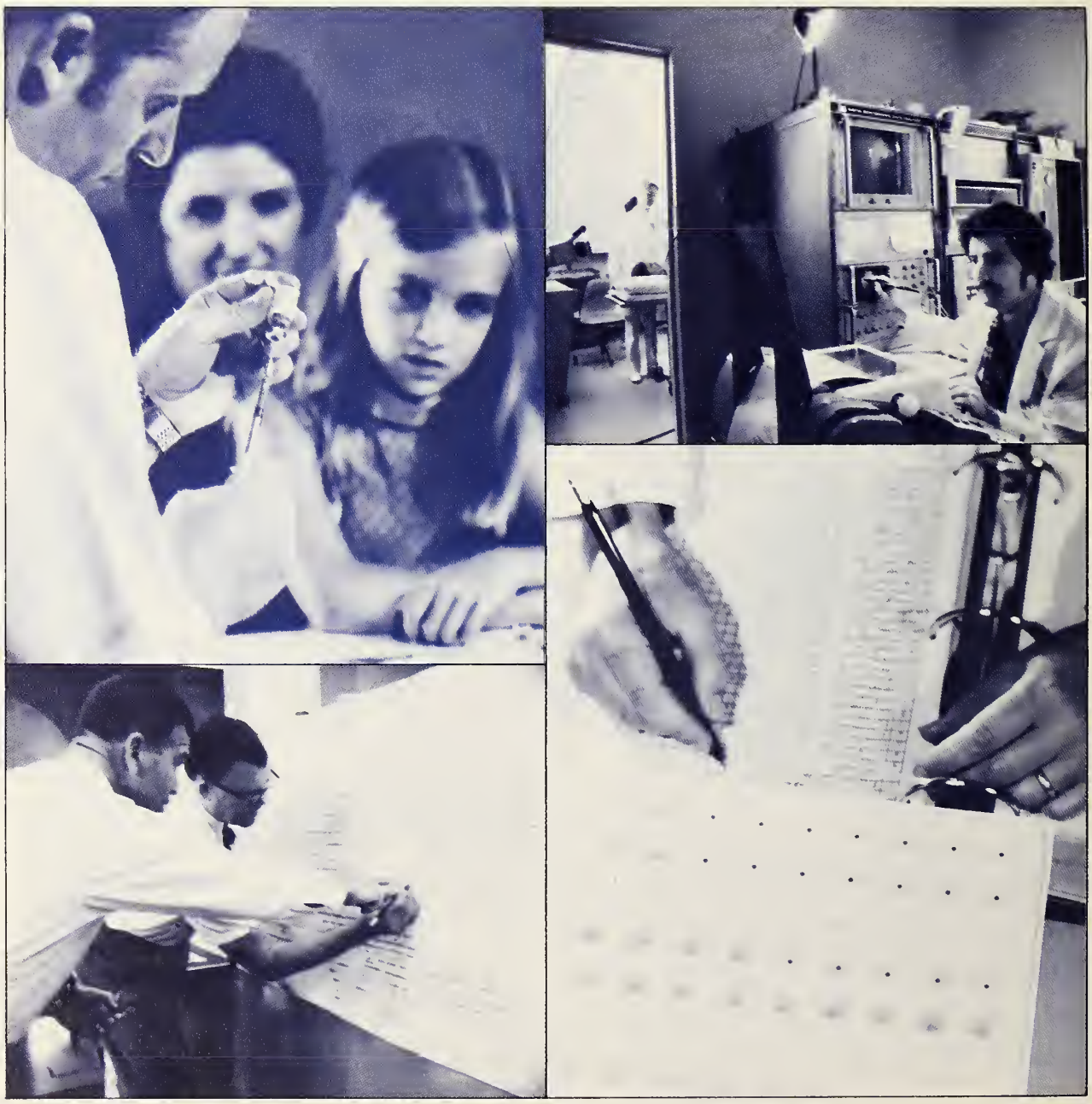
In the shunting operation, the surgeon uses a catheter (a long slim tube whose tip is in one of the fluid filled cavities of the brain) to drain fluid from the head into the heart or abdomen where it is reabsorbed in the blood stream.

Although this operation is still considered the most effective means of treating hydrocephalus, it has one major drawback. As the child grows, the procedure must be repeated at age 18 months, 4 years, 10 years, and sometimes later to lengthen the catheter. Each time, scarring left by previous surgery adds to difficulties which contribute to the low rate of survival among children undergoing the procedure.

To meet this challenge, Drs. Milhorat, McClenathen, and Hammock developed the new shunt that unwinds as the child grows. The shunting procedure is carried out as before except that an extra 8 inches of catheter coiled in a small plastic pouch is implanted in the patient along with the drainage catheter.

The new shunt permits a one-step operation for hydrocephalus. Although early results with this procedure are encouraging, many years of critical evaluation will be necessary before adequate assessment will be possible.





### New Virus for Chickenpox Vaccine

A new virus has been discovered at the Delta Primate Research Center in Louisiana which bears a close resemblance to the chickenpox virus—varicella.

The discovery of the new virus, now labeled Delta Herpesvirus (DHV), may eventually lead to the development of a chickenpox vaccine to conquer one of the few remaining uncontrolled childhood diseases.

One of the major obstacles to developing a vaccine against the chickenpox virus has been the lack of an experimental animal for laboratory studies. The solution to the problem is now in sight with the surfacing of this chickenpox-like disease in Patas monkeys at the Delta Center, which is supported by NIH's Division of Research Resources.

Chickenpox is the most common reportable disease of children in the United States. During the first half of 1975 over 110,000 cases were reported, and the actual number could be much higher. Few people reach adult life without becoming infected by chickenpox. Although usually benign, it can result in more serious disease, such as encephalitis in children, or pneumonia in adults. It is also a serious problem in persons with hematopoietic and lymphatic malignancies such as leukemia, Hodgkin's disease, and lymphoma. Patients who have inadequate immunological defenses are particularly vulnerable. Under these conditions, the disease is often fatal.

The DHV virus caused two outbreaks in Patas monkeys. The disease resembled a more severe form of chickenpox. Antigenically, the virus closely resembles, or is identical to, varicella virus. Like varicella, DHV is a cell-associated virus which means it is difficult to recover large quantities of the virus free from the cells in which it grows. Other biological features, however, make it distinguishable from the human chickenpox virus.

The disease produced by DHV in Patas monkeys can be termed as a monkey form of chickenpox and will be useful as a model of human varicella. Because DHV stimulates antibody that affects varicella virus, it may have some potential usefulness as a vaccine against chickenpox. Any anti-varicella vaccines which will be developed can be tested against the experimental disease in monkeys.

This varicella-like disease of monkeys can also be used to evaluate antiviral drugs for their potential in preventing or curing varicella infections. Some laboratory evidence suggests that DHV may be used in tests to measure human immunity to chickenpox.

Dr. Ambhan Felsenfeld is the virologist who heads the group engaged in transmission studies of the new virus.

The development of the Patas monkey as the suitable laboratory animal model will enable researchers to study the disease in detail, perfect methods for laboratory growth of the virus, and develop a successful vaccine.



### Dietary Treatment of Renal Failure

A dietary supplement in the form of keto-analogues of essential amino acids that can postpone the need for dialysis in some cases of renal failure and lengthen the period between dialysis or temporarily take patients off dialysis in other cases, has been demonstrated by clinical researchers at the Johns Hopkins General Clinical Research Center (GCRC).

Clinical trials conducted by Drs. Mackenzie Walser and Willima E. Mitch at the DRR-supported Inpatient and Outpatient GCRC have established that the provision of a dietary supplement in the form of keto acids decreases the load of nitrogeneous waste required for excretion by the kidney.

The researchers determined that these substances could serve as building blocks for protein, and that under these conditions there is a marked conservation of nitrogen by the body.

It is estimated that approximately 60,000 people die each year in the United States from renal failure. Most of these people could be helped by hemodialysis (an artificial means of removing waste from the blood normally removed by the kidneys when they are functioning properly).

At present, about 16,000 patients are receiving dialysis, which is expensive (approximately $125 per treatment), inconvenient, and time-consuming.

Because metabolism of nitrogen in the body begins with dietary intake of protein and ends up with renal excretion of urea, the Johns Hopkins scientists have concentrated their efforts in improving the efficiency of the transamination process in the body. Transamination is actually a recycling process wherein nitrogen (in liver and muscle) is transferred from nonessential to keto acids so as to form essential amino acids. This process serves to adjust levels of individual amino acids to tissue needs.

This treatment in most cases has postponed the need for dialysis by renal patients for months, the clinical scientists report, by reducing urea production—and in some cases by slowing down renal deterioration.

In a most recent GCRC outpatient study, seven renal failure cases were treated with the keto acid diet. With the use of this therapy, six of the patients were maintained for an average period of 6 months before dialysis had to be instituted or reinstituted. The seventh patient remains completely off dialysis after more than 1 year.



None of the group developed any of the usual signs of renal incompetence or uremic poisoning, including peripheral neuropathy, premature atherosclerosis, renal osteodystrophy, or lowered bone density. Three of the seven had shown reduced nerve conduction velocity prior to treatment. This improved during keto acid therapy in two of the three. Three patients also showed a reduction of previous high plasma triglycerides to values within the normal range.

The keto acids used in these clinical tests were synthesized at the Johns Hopkins University School of Medicine. The studies at the GCRC were also funded by a grant from the National Institute of Arthritis, Metabolism, and Digestive Diseases and a contract under their Artificial Kidney/Chronic Uremia Program.

### New Cell Reconstruction Technique

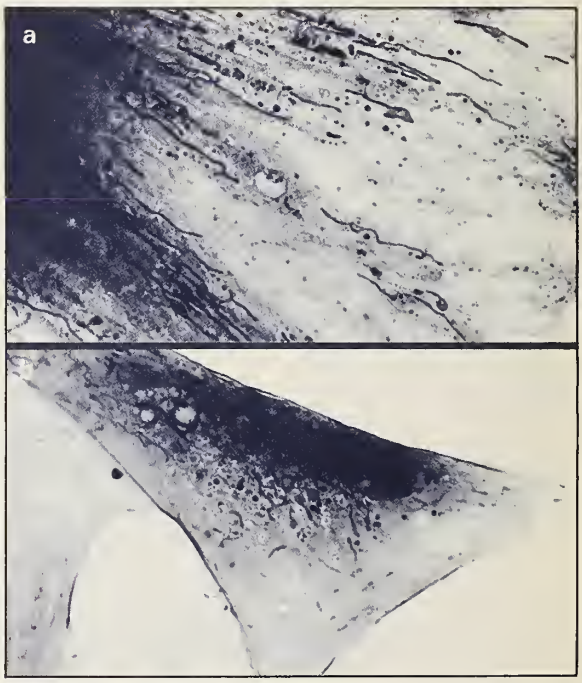
A new cell reconstruction technique, enabling cell biologists to mass produce millions of cells in different combinations of nuclei and cytoplasms has been developed by scientists at the University of Colorado (CU) at Boulder. The new technique opens up many areas of research, including prospects for generating haploid cells (cells with only half the number of chromosomes in body cells), and for investigating the ability of cells to differentiate into nerve cells, muscle cells, and the rest of the specialized types of cells which make up man and other multicellular life forms.

Laboratories throughout the United States have now adopted the new technique and are using it for their studies.

The CU method of cell reconstruction was evolved with the aid of the million-volt electron microscope which is supported by the DRR. The microscope at CU is one of the only two existing million-volt electron microscopes in the U.S. being used for biomedical research. The other one is located at the University of Wisconsin.

The great penetrating power of the million-volt electron beams and the reduced beam damage permits the investigator to obtain sharp 3-D images of how cells are constructed. It is now possible to view whole intact cells with the various subcellular components clearly resolved. For example, CU scientists report that they have found large molecules within the nerve-muscle membrane system of mice and chickens never previously seen before.

Drs. Keith R. Porter, George Veomett, David M. Prescott, and Jerry Shay comprised the research team which successfully developed the new cell reconstruction technique.




Million-volt electron micrographs of whole and enucleated (nucleus removed) rat muscle cell. (a) shows the thinner margins of a whole muscle cell. The nucleus is not shown but is toward the upper left. (b) is an enucleated muscle cell. The removal of the nucleus makes the cell much thinner and accessible to study.

Using a special strain of mouse cells (L929), the CU scientists first treat the cell with a compound called *cytochalasin B*, which frees the nucleus to move to the outer edge of the cell. At this point, only low centrifugal force is needed to pull the nucleus out of the cell and break the thin thread between the nucleus and the cytoplasm. Thus is formed the karyoplast (a body containing the nucleus and a small amount of cytoplasm), and the cytoplast (a body containing the remainder of the cytoplasm).

Two such treatments on different types of cells provide scientists with karyoplasts from one set

of mouse cells and cytoplasts from another set. When these two parts of the cells are exposed to an inactive virus, they re-fuse, and a mass of hybrid cells is produced.

The success of the fusion is tested by feeding a radioactive substance to one group of cells in culture, and tiny latex spheres (a plastic material similar to that added to latex paint) to another set of culture cells.

The radioactive substance is known to be deposited in the nucleus, and the large latex spheres to be taken up by the cytoplasm. When most of the resulting hybrid cells had both, CU scientists knew that the fusion had been successful. The resulting cells remain alive and continue to divide, thus proving that they are not impaired by the process.

### Artificial Intelligence SUMEX-AIM Resource

The Stanford University Medical Experimental Computer (SUMEX-AIM) has been established to provide a national shared computer facility for medical research. Funded by NIH's Division of Research Resources, this facility will concentrate on the application of artificial intelligence in medicine.

Directed by Dr. Joshua Lederberg, professor and chairman of the Department of Genetics, SUMEX-AIM is an innovative effort to help biomedical scientists meet today's research requirements and to explore computer applications in many health fields, ranging from basic research to bedside care.

At present, SUMEX-AIM consists of a powerful PDP-10 computer available to approved users throughout the U.S. over a computer communication network on a time-shared basis.

Artificial intelligence is a part of computer science concerned with the symbol-manipulation processes that produce intelligent action. Scientists in this field utilize the computer to reach decisions and solve problems through symbolic analysis and reasoning.

In medicine this approach is being applied initially in medical diagnosis, planning of therapy, and the interpretation of data from advanced chemical structure studies.

Some major artificial intelligence projects currently in progress are:

CASNET. A group of computer scientists, led by Dr. Casimir Kulikowski of Rutgers University, is developing computer-based consultation systems for diseases of the eye in collaboration with Dr. Aran Safir, opthalmologist, at the Mount Sinai School of Medicine. CASNET, a network of collaborators for computer diagnosis and treatment of glaucoma, has been established. The computer system, which includes an elaborate pathophysiological model of the disease, is being tested in eye centers at the Mount Sinai Hospital and Medical Center, New York; Washington University, St. Louis; and the Johns Hopkins University Hospital.

DIALOG. A diagnostic project under the direction of Drs. Harry Pople and Jack Myers of the University of Pittsburgh, the DIALOG system deals with the general problem of diagnosis in internal medicine. Its medical data base encompasses about 50 percent of the major diseases in internal medicine.



MYCIN. This is a computer-based-consultation-in-clinical-therapeutics project directed by Dr. Stanley Cohen, associate professor and head of the Division of Clinical Pharmacology at Stanford University. MYCIN attempts to model the processes medical experts go through in selecting therapy for patients with bacterial infections.

DENDRAL. This program is aimed at assisting the biochemist interpret molecular structures from mass spectral and other chemical information. It is conducted at Stanford University under the leadership of Drs. Joshua Lederberg, Edward Feigenbaum, and Carl Djerassi.

X-RAY CRYSTALLOGRAPHY. Protein crystallographers Drs. Joseph Kraut and Stephen Freer at the University of California, San Diego, are using the SUMEX-AIM facility as the central repository for programs, data, and other information of common interest. The objective of the program is to apply problem-solving techniques, emerging from artificial intelligence research, to determine the three-dimensional structure of proteins.

### The Armadillo for Leprosy Skin Testing

The development of the nine-banded armadillo as the key laboratory animal model for leprosy research (Hansen's disease) has contributed significantly to recent breakthroughs in skin testing for human leprosy.

Leprosy affects an estimated 15 million persons in the world, with heavy concentration in tropical zones. The skin test is used in classifying the type of leprosy in afflicted patients.

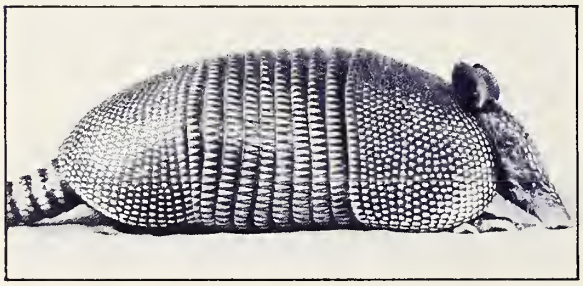
Researchers at the Gulf South Research Institute

in Louisiana report that lepromin prepared from armadillo bacilli is comparable in quality to lepromin prepared from human bacilli. Human bacilli are not always available.

The sole source of significant amounts of armadillo leprosy bacilli, Gulf South has recently been designated as a collaborative laboratory of the World Health Organization.

Dr. Eleanor E. Storrs, director of the Department of Biochemistry, is the prime motivator of armadillo research and colony development—now the largest in the world—with support from the Division of Research Resources.





The low skin and body temperature of the armadillo is one of the major factors which makes the animal suitable for leprosy research since the leprosy bacterium requires a low temperature for optimum growth.

The armadillo's span of life is from 12 to 15 years, giving researchers a longer period to study the progressive form of the disease. In man, the estimated leprosy incubation period averages 3 to 5 years. It is now known that the leprosy organism invades the bone marrow and lungs in the armadillo.

Medical scientists previously had developed something resembling progressive leprosy in mice following removal of the thymus gland and destruction of bone marrow by X-ray. However, mice live only about 2 years, and there was a need for an unaltered animal with a longer life span.

Successful inoculation of lepromatoid leprosy in the armadillo was reported in 1971 as the result of collaborative study by Dr. Storrs and Dr. Waldemar F. Kirchheimer of the U.S. Public Health Service Hospital at Carville, Louisiana. Gulf South researchers have since found that leprosy-induced armadillos have extremely high bacilli levels. A single armadillo has yielded sufficient bacilli to prepare 1,500 liters of standard lepromin—enough for 15 million lepromin skin tests.

The unusual physiological characteristics of the armadillo lend themselves to several other important areas of biomedical research. The animal is apparently the first laboratory model found for studies of *Buruli ulcerans* (a type of skin ulcer). The armadillo is also a natural host for Chagas disease (a parasitic disease prevalent in South America), and a wide variety of rickettsial diseases.

Current collaborative programs with use of the armadillo include the establishment of a World Lepromin Bank, and the development of a vaccine against leprosy.

### Rapid Access to Health Science Audiovisuals

The National Library of Medicine is concerned with biomedical information in all its forms—not just the traditional print medium represented by books and journals. One form of increasing importance is audiovisual. Educators confronted with the daily task of instructing students in the health sciences, and practitioners seeking to keep their professional skills up to date frequently ask: "Where can I get high quality audiovisual instructional materials?"

A new service of the National Library of Medicine, begun on an experimental basis in 1975, holds the promise of an answer to this question. The service is AVLINE (Audiovisuals On-Line), an on-line inter-active retrieval system based on the existing MEDLINE network.

The user of AVLINE is able to search a rapidly growing (but still relatively small) data base of peer reviewed audiovisual teaching packages and select those that he needs. The "on-line" feature of AVLINE means that the user can "converse" with the computer—asking successive questions until he identifies precisely those audiovisuals that he needs.

AVLINE is available at the more than 350 health science libraries that have MEDLINE terminals connected to the National Library of Medicine's IBM 370/158 computers. As with MEDLINE, which provides journal article references, AVLINE retrieval is based on a thesaurus of medical subject headings under which the materials are indexed or cataloged. For AVLINE, however, there is also other information pertinent to the use of audiovisual materials. For example, a user may be limited to 16mm motion picture projection or ¾-inch video tape playback. There are more than 60 data elements or descriptors in the AVLINE system, such as title, abstract, audience level, playback medium, author, source, and price. The computer can be commanded to print out any or all of these data elements for each citation retrieved.

The Library, through its component in Atlanta, Georgia, the National Medical Audiovisual Center, has over the last several years been cataloging the vast array of health science audiovisual materials now available. These biomedical films, video tapes, slide packages, etc., are now being reviewed for quality with the assistance of the Association of American Medical Colleges and the American Association of Dental Schools.

The process is comparable to refereeing scientific papers for journal publication. The AAMC and AADS have established cooperative agreements with over 60 professional societies that have, in turn, designated more than 320 distinguished scholars from their membership to act as reviewers. Panels composed of different disciplines rate audiovisual instructional material for content, accuracy, currency, and relevance. To reduce the size of the task, a prescreening eliminates items lacking sufficient technical quality and those not available for widespread distribution. Of the 3,200 audiovisual packages reviewed by June 1975, over 2,300 have been rated as acceptable for the AVLINE data base.



Review is a never ending task. Instructional packages must be re-evaluated as factual information and interpretations evolve through research. Those still current upon re-evaluation are retained while others will be dropped from the file. New productions becoming available must, in turn, meet even higher standards of instructional value as more is learned about designing for effective learning.

Medical librarian uses computer terminal to retrieve citations on instructional materials in AVLINE

It is encouraging that more and more of the newer instructional materials are meeting the high standards. In the past, audiovisual materials were used mostly to support lectures. Newer items are oriented toward self-instruction and include measurable objectives for student performance. Some of the recent audiovisuals are constructed in small modules or instructional segments, so they serve several different audiences and their particular learning objectives. An accompanying printed guide

provides instructions on the use of the materials and also furnishes items for performance feedback. These learning materials serve well in libraries, traditional lectures, and laboratory teaching. In some instances they are designed to accompany computer-based instructional materials and take advantage of simulation devices for the development of special skills.

The data base will be steadily expanded in 1976 to include audiovisual instructional materials from all the health sciences. On the basis of the responses received so far, users are convinced that AVLINE will serve a serious communication need in the future.



### Toxicology Data Bank

Ten years ago, a panel of the President's Science Advisory Committee met to consider the problem of increasing health hazards posed by human exposure to chemicals and other harmful substances. One result of this concern was the establishment of a Toxicology Information Program at the National Library of Medicine. At the time of its creation the focus of attention was on the potential adverse effects of new drugs. The thalidomide experience was fresh in people's minds. Since that time, the emphasis has shifted from compounds that cause acute adverse reactions to those that have long-term deleterious effects. No matter what the substance, however, or how it does its harmful work, an effective toxicological information program must provide methods to make the results of toxicological investigations quickly, accurately, and widely available to those who perform research, make regulatory decisions, and monitor the environment. It must help to prevent duplication of research which would waste limited funds and manpower.

The most important accomplishment of the Toxicology Information Program, reported in *Research Advances, 1975,* was TOXLINE (Toxicology Information On-Line). TOXLINE continues to enjoy wide acceptance in the scientific community; during the year ending June 30, 1975, over 22,000 searches were done on this data base from terminals located in medical libraries, research institutions, and industrial organizations around the country. As nationwide concern about the health effects of harmful substances continues unabated, however, another data base being developed by the National Library of Medicine will become increasingly important to the scientific community: the Toxicology Data Bank.

Since on-line bibliographic searching has proven to be successful and highly versatile, it was natural to consider data ("fact") retrieval as a candidate for an on-line interactive system similar to TOXLINE, but containing information about the properties of hazardous chemical compounds. This system, called the Toxicology Data Bank, will have some of the characteristics of a "handbook," but unlike a printed handbook, the data retrieval system will allow the user to coordinate attributes of compounds in any way necessary to answer a particular question.

Information for the Toxicology Data Bank is being extracted from evaluated sources such as textbooks, reviews, criteria documents, and the files of cooperating organizations. The data from these sources are being represented in the Toxicology Data Bank as either numerical values or verbal descriptions, depending on the subject, with the sources clearly identified. Where possible, the descriptions themselves are also indexed using a controlled vocabulary.

Data elements in the Toxicology Data Bank include: substance identification; chemical/physical properties; animal toxicology including teratogenesis, mutagenesis, carcinogensis, and lethal dose values; human toxicology; metabolism; pharmacotherapy; overdose treatment; drug interference; drug interactions; transportation hazards; manufacturing information; and environmental hazards. Users will be able to search the data bank by asking for specific data fields either singly or in combination, or by specific data items without identifying the data fields.

The Toxicology Data Bank has been developed by using lists of hazardous substances prepared by Federal agencies and other organizations. By the fall of 1976, it will contain about 1,000 compound records available for searching at any of the 400 MEDLINE and TOXLINE terminals throughout the United States.

### Atlas of Protein Structure

The Division of Computer Research and Technology (DCRT) provides NIH with central technological resources and scientific expertise in computer science. The Division combines a strong computer center and programming capability with several multidisciplinary laboratories which collaborate with NIH scientists on solutions to specific biomedical problems.

For example, the computer is being used to assist NIH scientists in studying protein structure and function. Biochemists have long recognized the importance of protein molecules, such as those which comprise the structural elements of muscle and the enzymes which facilitate chemical processes in living organisms.

The development of X-ray crystallographic techniques and computer technology over the last decade has made it possible to visualize the explicit structure of these complex molecules. Using several computer techniques, the crystallographic data are reduced mathematically, then organized in a computer file and finally projected in a three-dimensional dynamic graphic display which allows the scientist to examine interactively the computed structure both globally and in any level of detail.

Since these computer display systems are very costly (over $100,000), the challenge has been to develop a system which would allow greater access to three-dimensional views of protein molecules by the biomedical community, thereby enhancing the research process. Toward this end, the Global Atlas of Protein Structure on Microfiche (GAPSOM), developed by Richard J. Feldmann of the Division exploits the potential of the less costly microfilm medium. This offers a substantial reduction in the cost of reproducing textual and graphical information. More importantly, its flexibility allows any information or manipulative capability of the computer to be diverted onto the micro medium.

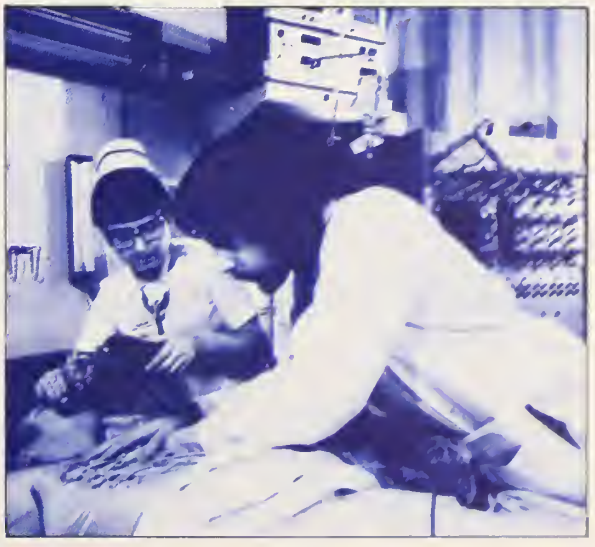
A microfiche generation package developed by Charles T. Bacon, also of the Division, takes the protein structure data and by computer-controlled microfilm output, produces selected views of a protein molecule on microfiche. The microfiche medium is so inexpensive that there is virtually no limit to the amount of detail that can be generated. The atlas, therefore, can contain all desired details, calculations and significant projections of a structure. With proper indexing, it is easy to locate any specific detail view or calculation of interest.

One advantage of an interactive computer display system is its ability to rotate a molecular image, allowing perception of depth in the image. The development of two-image stereo techniques, however, has obviated the necessity of image motion for depth perception. GAPSOM takes advantage of these capabilities—the use of a stereo device attached to the microfiche viewer allows three-dimensional viewing. Since the cost of the global atlas, the stereo viewing box, and the microfiche viewer is about $300, the development of this technology has enabled the relatively inexpensive dissemination of basic scientific information.

Specifically, GAPSOM will provide an aid to protein crystallographers in their structure determination problems; it will help general biochemists to understand the structure and function of proteins; and it will be useful to students and teachers in their understanding of protein structure and of progress in protein research.

### Evaluation of Electrocardiogram Computer Programs

During the last 10 years, several computer programs for the interpretation of electrocardiograms (ECG's) have been developed and are now available for routine use. Approximately 4 million ECG's were analyzed by computer in 1974 (compared to 1 million in 1972) in the United States and Canada. However, the reproducibility, diagnostic accuracy and acceptability of ECG computer programs have been subjected to considerable debate. Disagreements between ECG interpretations by physicians and computer programs have occurred, and have varied widely depending upon the specific computer program and the method of analysis used.



Drs. James Bailey and Martha Horton at DCRT and Dr. Samuel Itscoitz of the Cardiology Branch, NHLI, have developed a method for the evaluation of ECG computer programs which avoids major pitfalls of previous studies. The method requires the collection of a large population of ECG's which contains a significant variety of abnormal tracings. Each ECG is read separately by each of three cardiologists, none of whom is aware of the computer "interpretation" or the previous reading at the time of his review. If the cardiologist disagrees with either another reader or the computer program, he must defend his reading (or modify it) by the application of specific, fixed diagnostic criteria. The end result of this process is the minimization of human variability as a factor in the test—i.e., the readings of all cardiologists in the test ultimately agree.



The remaining disagreements between the cardiologists' reading and the computer program's "interpretation" are separated into those resulting from criteria differences and those resulting from programming errors, namely, pattern recognition failures, mismeasurements, and/or deficient program logic. If the program errors are common, then the program has serious deficiencies independent of its diagnostic criteria and its use in a clinical setting is not recommended.

If, however, the frequency of program errors is low, then the proportion of diagnostic criteria differences becomes an important variable in deciding whether to implement a given computer program in a specific insititution. If the proportion of criteria differences is high, then the program will be unacceptable to the clinicians who might use it, unless it can be shown in an independent study by non-ECG data such as echocardiogram, contrast angiography or autopsy measurements, that the criteria used by the program in question are, indeed, more accurate than those used by clinicians in their setting. If both the percentage of criteria differences and the program error rates are low, then implementation of such a program may be warranted.

### System Aids in Mathematical Modeling

MLAB (modeling laboratory) is an interactive system used in the interpretation of biomedical research data. It is used primarily for mathematical modeling, which is the attempt to find values for parameters which occur in a mathematical function designed to "describe" some situation. MLAB calculates the parameter values that make the function best "fit" observed data values. Such curve-fitting is a convenient way to view the results of experimental data and is also useful in determining values of otherwise unmeasurable parameters—for example, the rates of decay.

MLAB was designed by Dr. Gary Knott at DCRT and Douglas Reece, based on a curve-fitting routine programmed earlier by Richard Shrager (DCRT). An outstanding feature of the program is its interactive graphic interface with the user. A user gets what he needs by issuing a sequence of simple and direct commands. MLAB accepts a command from the user's terminal, obeys it, and then waits for the next command.

By using MLAB, a researcher can define models and specify data, fit curves, solve differential equations and produce high-quality graphic plots of functions and data on a cathode ray tube display. A finished graph for publication can be produced on paper by means of an MLAB command which invokes the aid of a Calcomp plotter.

This program has been used in a variety of projects at the National Institutes of Health. Dr. Marc Lewis of the National Eye Institute has used MLAB extensively as an analytical tool to process and interpret ultracentrifuge data. Specifically, he uses the system to generate data describing a specific kind of macromolecular association of interest to him. With the aid of the program, he adds random noise to his data. MLAB is then used to fit the generated data (with error) to an experimentally-determined curve, and the fitting parameter is compared to the data-generating parameter to see the effect of random error and statistical error on the parameter.

MLAB has also been useful to Dr. Jack Cohen at the National Institute of Arthritis, Metabolism and Digestive Diseases in his application of nuclear magnetic resonance spectroscopy to proteins. It proves particularly helpful in the analysis of titration curves. He performs a titration to observe whether a change in the acidity (pH) level of the test produces a chemical shift difference and an accompanying change in the resonance spectrum of the protein. MLAB provides an aid to plotting the data, and, subsequently, fitting different curve models to this data. The use of computer curve-fitting facilitates the determination of the continuities of the titration curves.

### Rapid Scan NMR Spectroscopy

Many atomic nuclei evidence a spinning

behavior, which provides them with magnetic moments. Scientists have discovered that, by applying an external magnetic force to the molecules within which these nuclei reside, and subsequently introducing radio frequency electromagnetic energy, it is possible to determine a sharply defined frequency at which energy is absorbed by the nuclei, causing them to deflect measurably. This phenomenon is known as nuclear magnetic resonance (NMR).

The NMR spectrum is useful in determining the chemical environment of specific nuclei, since, for specific nuclei, this phenomenon occurs only at a well-defined frequency. It is an especially powerful help in determining molecular structure, since the molecules are not disturbed in the process.

NMR spectra are usually produced by either of two methods. The first, developed in the 1950's, applies a continuous wave (cw) frequency, slowly scanning one radio frequency at a time, to determine resonances of a sample. Another method, which came into use in the seventies, applies a short intense radio frequency pulse to simultaneously excite the whole resonance spectrum of a substance. By mathematical analysis procedures, it is then possible to determine the spectrum at different frequencies.

In both the continuous wave and pulse methods, resultant spectral response patterns are recorded in the memory of a small computer. Repetitive scans are performed and these traces added to computer memory in order that subsequent analysis will provide an improved signal-to-noise ratio.

Drs. James Ferretti of DCRT and Edwin Becker of NIAMDD have collaborated in refining a new technique introduced by Dr. Josef Dadok in his work at Carnegie Mellon University under an NIH grant. This rapid scan method is a fast passage variation of the continuous wave technique, although mathematical manipulations must be performed to extract the slow passage spectra.

Rapid scan provides several advantages over the cw or pulse methods. A major benefit is seen in work with biological molecules (for example, proteins) in water. Since rapid scan is applied sequentially, a portion of the spectra of specific interest can be scanned; in pulse NMR, where the whole spectrum is obtained simultaneously, the portion of the spectrum of interest tends to be dominated by the large resonance peaks of water.

Additionally, a conventional cw spectrometer can be easily and inexpensively converted to a rapid scan operation without the necessity for expensive, high power pulse apparatus. This is a distinct advantage, since the efficiency of the rapid scan method is comparable with pulse methods and is many orders of magnitude greater than the conventional cw scan.

### Environmental Poisoning of Rhesus Monkeys

During the past 3 decades, a remarkable increase in use of primates, particularly rhesus monkeys, in biomedical research has occurred because of their physical similarity to humans. They are invaluable for studying many infectious diseases and behavioral problems and for testing the safety of drugs and biologicals. Until recently, most of these primates were wild-trapped, properly quarantined animals. Now, foreign countries supplying primates for research purposes have placed limitations on exports severely limiting their availability to the research community.

This prompted the Division of Research Services to establish breeding colonies within the United States and its territories. A breeding colony, primarily rhesus monkeys, was installed at Perrine, Florida. Facilities for housing the primates, consisting of concrete rectangular runs enclosed by cyclone-type galvanized fencing, are conventional for the mild subtropical Florida climate.

After a number of months, it became apparent to the clinical veterinarian at the Perrine facility that a considerable number of monkeys had developed an unthrifty appearance, a loss in weight and generalized loss of hair. Some animals developed conjunctivitis, an inflammation of the eyelid, with excessive tearing also accompanied by marked facial swelling. In the latter stages of the disease, a persistent diarrhea often unresponsive to treatment developed which eventually resulted in death.

Postmortem examination of these animals revealed conjunctivitis and distorted cellular changes such as squamous metaplasia, a scaly abnormality of the tear ducts, and marked loss of hair. Approximately 25 percent of the animals had developed a severe thickening of the stomach lining. The most severely affected animals also had similar, but much less severe changes in the colon. Clinical chemistry and blood studies revealed only a moderate anemia and neutrophilia, an increase in certain white blood cells without a significant increase in the total number of white cells. The functional studies and the pathological picture observed is consistent with chemical poisoning due to halogenated hydrocarbons, in particular polychlorinated biphenyl compounds (PCB) used in lubricants and detergent motor oils.

Through the use of electron gas chromatography and mass spectrophotometry to examine tissues taken from live monkeys showing moderate to severe weight loss, hair loss and conjunctivitis, significant toxic levels of PCB in the body tissues were revealed. In some cases the levels were very high. Samples of concrete from the floors of the monkey runs also revealed the presence of PCB's, and attention was directed to the sealer used on the concrete. The compounds revealed in the tissues, the concrete, and the concrete sealer have similar retention times on gas chromatography columns to commercial PCB compounds.

Plans are now being made to reproduce the disease experimentally with the concrete sealer. Appropriate measures are being taken to protect the monkeys at the Perrine facility from further exposure. More care in the use of such compounds and advance testing of materials used in animal facilities are indicated to increase the reliability of research animal models.

Recognition of this environmental poisoning in monkeys is a warning of potential danger to human health posed by the widely used halogenated hydrocarbons.

### Special Diets for Research Animals

Another critical aspect of reliable production and maintenance of high quality laboratory animals is under continuous study in the Division. The feed animals consume profoundly influences their growth, reproduction, behavior, and longevity. It is therefore essential that diets contain optimal nutrient concentrations for the various animal species. In a research community such as NIH, many special diets are required to

meet specific research objectives. For example, diets containing different levels of nutrients are used in food requirement studies, test compounds are added to feed formulations in toxicology experiments, and diets made from special ingredients are essential for maintenance of some exotic animal models.

Under direction of Dr. Joseph J. Knapka, DRS nutritionist, research is conducted to ascertain the nutrient requirements of various species of laboratory animals, special rations are formulated for specific research projects as requested by NIH investigators, and procedures are developed to insure the quality of the diets purchased by NIH.

A result of Dr. Knapka's research is development of "open formula" diets for rats, mice, rabbits, guinea pigs and non-human primates. An open formula diet is one where the amount of each ingredient used in the formulation is known and readily available. In contrast, a "closed formula" diet is a well kept secret of the company that manufactures and markets the diet under a trade name. In addition to its economy, the advantages of knowing the complete formulation of a diet fed to research animals include the ability to produce essentially the same product at different locations and to alter the nutrient concentrations to meet specific research requirements. The open formula diet developed for conventionally reared rats and mice is being used not only at NIH but at other research institutions throughout the United States. Recently, a committee of the American Institute of Nutrition selected this ration as the recommended "Standard Reference" diet for research involving these species.

Special diets have been developed to study atherosclerosis, clogging of the arteries, in swine and dogs. The basic rations were formulated with ingredients essentially devoid of fat. By designing feeding trials where various sources (animal or vegetable) and concentrations of fat or combinations of fat and cholesterol are added to the basic rations, investigators learn how these nutrients affect the development of atherosclerosis.

Data from an experiment with 288 pairs of inbred mice representing four strains and six experimental diets differing in crude protein and crude fat concentrations indicate that the various mouse strains differ in their nutrient requirements. Results also show the ratio of crude protein to crude fat in mouse diets may be more important to optimal reproduction than the concentration of each nutrient *per se*. Many of the inbred mice in great demand for various kinds of research are strains that do not reproduce very well. The studies may lead to the development of better diets for these mouse strains, thereby improving their reproductive performance.



### Blood Flow Measurement in Heart Disease

The Division's biomedical engineers have been applying their knowledge of fluid flow phenomena to cardiovascular disease affecting the heart and blood vessels, which is responsible for more than half of all deaths in the United States every year. In particular, arteriosclerosis (hardening of the arteries), and related diseases such as hypertension (high blood pressure) account for a major portion of cardiovascular deaths. One type of arteriosclerosis, known as atherosclerosis, is slow but progressive and starts as early as childhood. This disease is characterized by an accumulation of fatty deposits (mainly cholesterol) in the arterial wall. During later stages of the disease, a fibrous cap of scar tissue called plaque, which looks like a pearly grey mound, forms inside the artery. If it becomes blocked or ruptures due to weakness, a heart attack or stroke may result.

Our knowledge of how plaques initiate and grow is limited, but one consistent and peculiar feature of the disease is that it appears preferentially in large arteries and near arterial branches. These regions of the body are also associated with unusual blood flow patterns (hemodynamics), and so it is thought that hemodynamics plays some role in the initiation and localization of atherosclerosis. Specifically, the blood exerts a shear force on the very sensitive cells lining the inside wall of the arteries, called endothelial cells, in much the same way that a river erodes its banks. The nature of this shear force may be a primary factor in promoting the disease.

In order to test this hypothesis, it is necessary to know the magnitude of the shear forces at various locations in the artery. To accomplish this task, DRS engineers in collaboration with scientists in the National Heart and Lung Institute are applying proven techniques useful for the measurement of shear stress in pipes to the problem of shear measurements in arteries.

Since measurements in living arteries are very difficult, a plastic replica of an artery from a dog was fabricated by making a series of casts. Using this simulated flow model of an artery, an electrochemical technique was employed to measure shear stresses. Seventy five small wire electrodes were implanted in the wall of the model at precisely the locations where a knowledge of the shear stress was desired. The principle of this technique, using each electrode as a shear measuring device, involves an electrochemical reaction at the electrode surface which in turn is dependent on the rate of fluid flow (shear rate) over the electrode. The higher the shear rate, the greater the electrode reaction rate, and the larger the recorded signal. The magnitude of the shear forces can be calculated directly from this signal by using the appropriate mathematical equations.



The experiments have shown that some regions of arteries where atherosclerotic plaques develop are areas of high shear stresses which may actually erode or damage the cells lining the inner surfaces of circulatory organs causing the arteries to become vulnerable to deposit and accumulation of fatty substances (lipids) from the blood. Other regions, according to the experiments, are areas of fluctuating or turbulent flow patterns which are thought to be responsible for subtle changes in the structure of the arterial wall making them more permeable to lipids.

Though many questions still remain unanswered, the combined efforts of engineering and biomedical research on blood flow in arteries may give further insight into the cause and prevention of atherosclerosis.

## Conference on International Health Costs and Expenditures

There is growing concern about rapidly rising health costs and expenditures in many countries of the world. An examination of this world-wide phenomenon is especially appropriate for the Fogarty International Center, which serves as a focal point for the study and consideration of issues in international health.

On June 2-4, 1975, the Center convened an international conference on health costs and expenditures. The gathering attracted 120 participants who examined the economics of the health-care delivery systems in Belgium, Canada, Denmark, France, the German Federal Republic, the Netherlands, Romania, Sweden, the United Kingdom, and the United States. Dr. Robert Maxwell of Great Britain presented an introductory overview and Dr. Herbert E. Klarman of the United States summarized the discussions.

Many factors contribute to increases in the costs of health care: changes in the nature, range, intensity, and sophistication of delivered health services; labor costs; health financing mechanism; and the proliferation of hospital facilities. These factors differ in various countries, however, because of varying social, economic, and political conditions.

The conferees addressed themselves to questions regarding: the costs of receiving health care services; the costs of providing health care services; the health care components which contribute most to the increase in costs; public and private expenditures on health care services; the relationship between reimbursement mechanisms and costs and expenditures; changes in health care policy and their impact on costs, expenditures, and utilization; and the current trends in various countries.

Some governments are attempting to moderate both costs and the demand for services by the use of co-payment mechanisms; by using waiting periods to ration services; by controlling the number of hospital beds and the number of physicians; by reduction of physicians' reimbursements; and by the regionalization of health services.

The conferees agreed that a uniform concept of health costs is a basic need. Different concepts are used, for example, by the U.S. Social Security Administration and by the International Labor Office. It would be helpful, therefore, for each country and every international organization to adopt a common definition of health costs.

It was suggested that a more meaningful analysis would relate health costs and expenditures to the economic and social benefits received by individuals and by nations. A further suggestion was that a subsequent international conference could focus on the cost-benefit or cost-effectiveness of innovations in health programs.

### Deficiencies in Bleeding Disorders Identified

Hemophilia A and von Willebrand's disease are two inherited bleeding disorders that, when severe, can result in severe bleeding from even the most minor trauma. Recent research from the Clinical Center and the National Heart and Lung Institute (NHLI) has defined the molecular defects in these two diseases, paving the way for testing new methods of diagnosis and treatment. Treatment usually involves transfusion of plasma fractions to replace the factor(s) necessary for clotting. New methods for the concentration of factor VIII, a coagulation-promoting protein in plasma which is deficient in hemophilia A and von Willebrand's disease, have resulted in more efficient use of available blood resources. This has enabled more patients to receive factor VIII concentrates for therapy.

Although both hemophilia A and von Willebrand's disease are associated with reduced levels of factor VIII, they are quite different in other respects. Hemophilia A is carried by mothers who are symptom-free themselves but transmit the disease to half of their male children. Von Willebrand's disease can be transmitted from either parent to children of either sex. Another major difference is that platelets (blood cells that form the primary plug in clots) function normally in hemophilia; whereas in von Willebrand's disease they do not.

In trying to unravel the abnormalities in these two diseases, researchers isolated a highly purified factor VIII protein from normal persons and studied its biological functions. They found, in laboratory studies, that the normal protein can correct both the clot-promoting deficiency in hemophilia A and von Willebrand's disease as well as the abnormal platelet behavior in von Willebrand's disease.

The purified protein from patients with hemophilia was also studied. It had the same biochemical characteristics as the normal protein and could correct the platelet abnormality in blood from patients with von Willebrand's disease, but it was deficient in clot-promoting activity. Thus, it seems that synthesis of the factor VIII protein in hemophilia is normal except for the deficiency of clot-promoting activity.

The factor VIII protein in von Willebrand's disease, however, has shown a spectrum of abnormalities. Clinical Center investigators have identified three groups of patients with von Willebrand's disease. Patients with the most severe cases show no factor VIII protein in their plasma; thus, clot-promoting activity and platelet function are very abnormal. In patients with moderate von Willebrand's disease, bleeding problems are less severe. Factor VIII is present in reduced levels in these patients, and the protein itself is abnormal. A third group of patients with von Willebrand's disease has a factor VIII protein normal in amount, structure, and clot-promoting activity, but markedly deficient in its ability to correct the abnormal platelet function of the disease. The protein in these patients has a specific deficiency of carbohydrate (sugars) compared to either the normal or hemophilic protein.

NHLI-supported scientists at the Mayo Clinic have done studies in pigs that show that the incidence of atherosclerosis in pigs with von Willebrand's disease is markedly lower than that of a control group of animals. This suggests that although patients with von Willebrand's disease have a bleeding syndrome, they may be protected from the development of major vessel disease.

Further characterization of factor VIII has led to better understanding of the complex structure of this unique molecule. Several subunits appear to combine to form the very large circulating factor VIII molecule. One subunit of factor VIII is produced by endothelial cells in the interior of blood vessels.

### Improved Method for Isolating Platelets Developed

Platelets, the smallest particles found in the blood, are of paramount importance in blood coagulation (hemostasis), since they form the primary plug in the clotting mechanism. Platelet abnormalities can cause uncontrolled bleeding in diseases such as idiopathic thrombocytopenia, leukemia, and other forms of cancer.

Research has shown that platelets survive in the human circulation for a limited time. As they age in the body, they undergo biochemical and functional modifications until they are eventually removed from the circulation. However, research on platelet function and the aging process has been hampered by the inability to remove all the platelets from whole blood without distorting them. Present centrifugation methods for isolating platelets from whole blood are unsatisfactory because they do not remove all of the platelets in the blood and those removed are contaminated with plasma proteins. However, Clinical Center investigators have devised a method for isolating platelets from whole blood so that they remain functionally and structurally intact.

The new technique involves the addition of a polysaccharide (Stractan) to the centrifugation process. First, whole blood is centrifuged; then the plasma and platelets are centrifuged again with Stractan to isolate the platelets. The total platelet population is removed, and the platelets are not distorted.

Thus, for the first time, investigators are able to observe and study platelet aging as it occurs in the body. This provides new avenues of research in diseases where the platelet number or survival is decreased.

Investigators can study platelets from small amounts of whole blood and selectively study individual platelet populations in health and disease. This may be helpful in selecting platelets with the greatest hemostatic activity for use in platelet transfusion.

### Computers Used to Identify Bacteria

Each year scientists are developing more and more uses for computers in medicine. Data storage and retrieval systems have proved invaluable in both diagnosing and treating a wide range of illnesses.

Researchers in the Clinical Center recently developed a computer system that will help to accurately identify bacteria and thus facilitate proper diagnosis of bacterial infections.

This system is especially useful in identifying organisms seldom encountered. Such organisms may be erroneously identified in routine laboratory practice because the routine test battery is necessarily limited and the laboratory technologist is not always familiar with the characteristic patterns of rare organisms.

The system developed at the Clinical Center incorporates 38 test procedures used to identify 34 species— most of the organisms submitted to the laboratory for diagnosis. The biochemical results are compared with data stored in the computer, and mathematically manipulated to determine probability. The computer then prints out the most likely diagnoses in their order of probability. It also lists the test results that contradict these diagnoses as well as additional tests that might be useful in further establishing the diagnosis. This system serves as a powerful diagnostic tool for work with problem bacteria and is extremely valuable for teaching purposes.

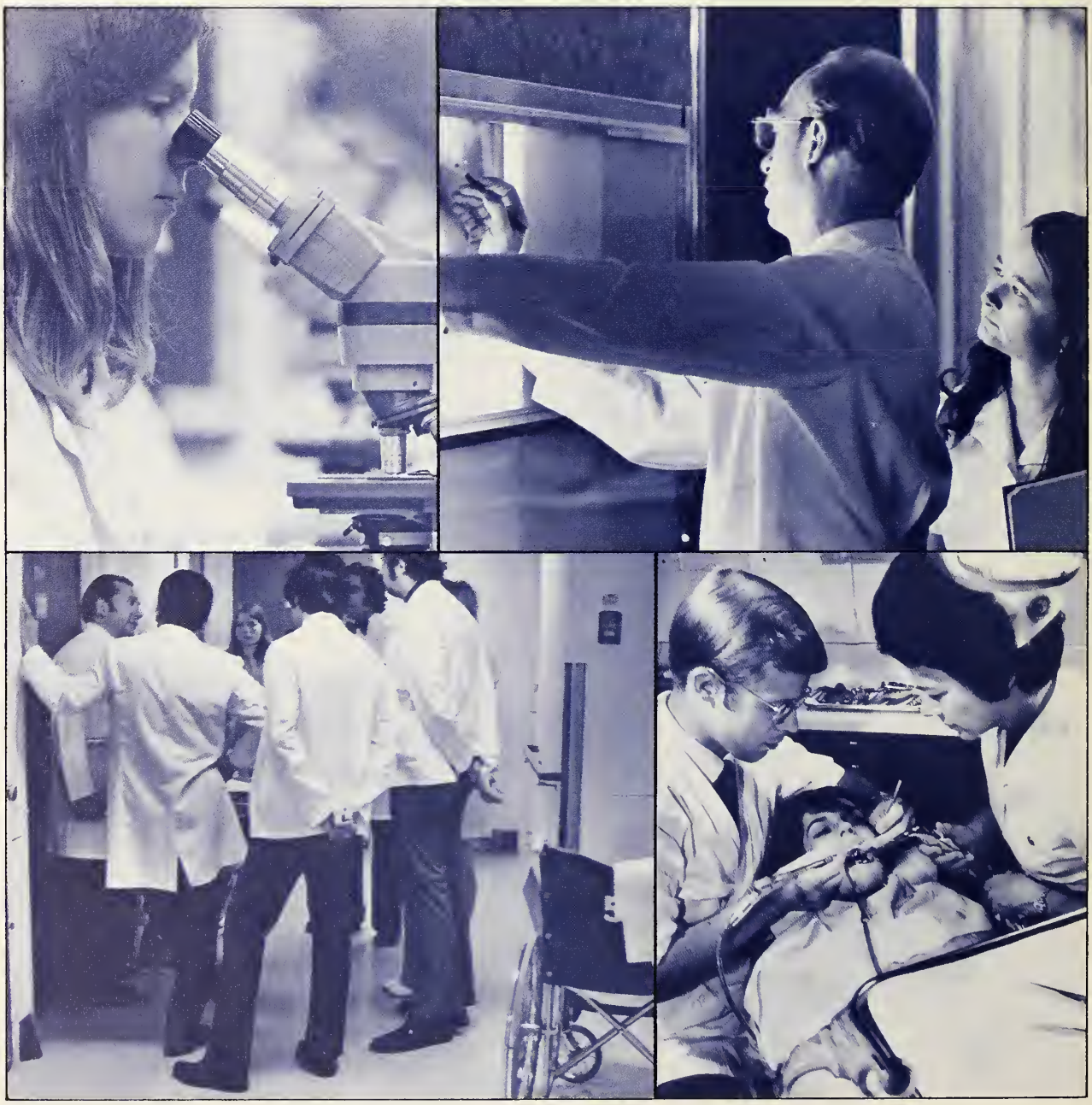
Another computer program was devised to identify bacteria according to their susceptibility to specific concentrations of antibiotics. Data collected in the past 7 years on over 35,000 isolated bacterial samples and their susceptibility to at least 11 antibiotics is contained in this data set. The information stored includes the laboratory number (which can be used to identify the patient), the diagnosis of the organism, the site of the organism in the body, and the minimal concentration of antibiotic required to inhibit the bacteria.

This information can be used to determine what antibiotic or combination of antibiotics at specified concentrations will be effective in treating bacterial infections.



Studies with these data indicate that organisms can be identified by their antimicrobial sensitivity. Although the system is not yet adequate for accurate laboratory diagnosis, more work is under way to reach acceptable standards. At present, the program is useful for quick preliminary identification of organisms for quality control in the laboratory, as a guide to changing bacterial resistance in hospitals, and as a way to study multiple drug resistance in bacteria.

Clinical Center investigators have developed a third computer system for use in epidemiologic studies. Biochemical data on certain types of bacteria are stored in the computer with information on patient identification and hospital location. Each month the computer puts out information on the distribution of bacteria by patient location. From this and other computer information it is possible to more rapidly investigate a local outbreak.



One of the unique features of the National Institutes of Health is the opportunity for training in clinical and laboratory investigation which is offered to young physicians, dentists, and Ph.D.'s through appointment as an NIH Associate or Fellow.

About 100 Clinical Associates, 50 Research and Staff Associates, 75 Staff Fellows, and 35 Senior Staff Fellows are appointed annually. In general, physicians come to NIH after an internship and a year or more of residency and Ph.D.'s after a year or two of postdoctoral study. The Clinical Associates participate in both clinical and laboratory programs while the others perform only basic research.

Located on a 300-acre campus in suburban Bethesda, Md., NIH is the largest biomedical research organization in the world. It provides an environment that nourishes productive medical research and offers training opportunities which are unparalleled elsewhere.

Close to 2,300 NIH staff members have doctoral degrees, including almost 1,100 with M.D. degrees, more than 1,000 with Ph.D. degrees, and about 50 with D.D.S. and 50 D.V.M. degrees. A significant number have more than one doctorate. Three Nobel Prize winners are on the campus, and many have won such honors as the National Medal of Science, the Rockefeller Public Service Award, and the Lasker Award. With the help of a staff of almost 2,000 technical personnel, NIH scientists are involved in over 2,400 different research projects which result in more than 3,500 published scientific articles each year. The budget for this intramural research effort is approximately $200 million. Almost all disciplines that contribute to new medical knowledge are represented. Many senior NIH investigators pride themselves on their availability for consultation and take great interest in young investigators, thus offering an unusual opportunity for the young scientist to associate with investigators who command towering respect in the scientific community.

An important resource for training in medical research is the Clinical Center, a 511-bed research hospital, which is at the core of the NIH campus. The Clinical Center provides a setting in which a young physician can participate in medical care, acquire new clinical skills, and carry out his own research in a laboratory conveniently located a few steps from the patient's room. Ancillary personnel and the widest range of bioinstrumentation are provided to enhance this patient-laboratory investigation arrangement.

Approximately 4,500 patients are admitted to the Clinical Center each year. To be admitted, a patient's specific disease or condition must be under active investigation by NIH physicians at the time of admission. The patient must have an understanding of his role in a research study and indicate his willingness to be a part of it. Since there is no charge for patient care, no patient is forced to leave for financial reasons. Nor is time a factor; indeed, long-term inpatient status or extended follow-up observations, or both, may be a paramount consideration for admission.



Clinical activities are similar to those at other teaching hospitals and include rounds, conferences, and other instructive exercises. The National Institutes of Health conducts a program of clinical electives for medical students in five clinical subspecialities: endocrinology and metabolism, oncology-hematology, immunology, infectious diseases, and clinical psychopharmacology. The medical students, who come from all parts of the country, share in the responsibility for patient care and add to the training of the Associate by putting him in the position of teacher as well as student. The proximity of the patients, students, associates, and scientists from many biological disciplines creates an unusual environment for clinical investigation.

While Clinical Associates are not, in general, in formal residency training programs (with the exception of pathology), some Associates do receive residency credit toward certification for board examinations. In addition, some programs have now been acknowledged as full fellowship equivalents. The best example of this is the program which has been developed in endocrinology and metabolism. In this program, the

Clinical Associate may rotate through clinical services in three different Institutes of NIH over the course of 1 year. This is then coupled with 1 or 2 years of laboratory training and has been accepted by the American Board of Internal Medicine as constituting a full fellowship training program in this area.



In addition, there are more formal academic programs which are open to all NIH Associates and Fellows. Evening courses are provided by the Graduate Program of the Foundation for Advanced Education in the Sciences. These include courses in behavioral and social sciences, biochemistry, chemistry, genetics, mathematics, statistics, physics, medicine, physiology, microbiology, immunology, languages, and general studies. Each week the NIH Calendar of Events lists about 30 formal seminars and programs which are open to all members of the NIH community, including the Combined Clinical Staff Conference which is published in the Annals of Internal Medicine. Finally, the Washington-Baltimore area contains five medical schools, several graduate schools with biomedical departments, as well as the Naval Medical Center, Walter Reed Army Hospital, and the Armed Forces Institute of Pathology. Some Associates and Fellows find time to pursue various programs at these nearby institutions.

While some stay on at NIH, the majority of Associates leave after a few years to join the staffs of universities as responsible investigators. Some have chosen to remain at NIH for lifetime careers of fruitful research. Whatever path he or she chooses, the young physician or Ph.D. cannot help being stimulated by the NIH environment that continually challenges while offering training and research opportunities difficult to match elsewhere. They are accepted by their colleagues and develop relationships based on reciprocal confidence and freedom of discussion.

To one recent visiting scientist, this was one of the most striking characteristics of NIH. Writing in the Neue Zurcher Zeitung, Dr. Jean-Marie Matthieu, a young Swiss pediatrician, commented that *"The most unusual feature of NIH is a number of implied rules, which are passed 'by diffusion' to every new colleague. The most important of these rules is the total readiness to cooperate encountered on every level. No director or Nobel Prize winner would ever refuse to discuss a problem. There are no internal barriers . . . NIH is undoubtedly a unique institution and an incomparable institution of continuing education for young researchers. It makes possible the maximum development of creativity in each scientist."*

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